ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF
MENTAL HEALTH

FISCAL YEAR 1973

PART II

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

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U.S. NATIONAL INSTITUTE OF MENTAL HEALTH

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Public Health Service National Institutes of Health

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ANNUAL REPORT

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research, and
Division of Biological and Biochemical Research

NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1972 - June 30, 1973

VOLUME II

INDIVIDUAL PROJECT REPORTS

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MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research, and
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NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1972 - June 30, 1973

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Serial No. M-OD-SW-1
1. Office of the Director, DCBR
2. Section on Social Work
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Conflict between the Parents of Schizophrenics

Principal Investigator: Carol F. Hoover, Social Worker

Cooperating Units: Section on Psychiatric Assessment, Family Studies and Personality Development in Adult Psychiatry Branch, DCBR.

Man Years:

Total:	.5
Professional:	.3
Other:	.2

Project Description:

Objective: To investigate levels of marital conflict and styles of coping with disagreement characteristically reported by the parents of schizophrenics, as compared to parents of maladjusted and parents of community young people. Conflict over children, particularly index patient compared to his siblings, is a particular focus. Marital dominance and perceived couple interaction are also studied.

Method Employed: A 70-item card sort is administered to each couple.

Major Findings: A total of 154 individuals have been tested, either at the N.I.M.H. or their own homes. These included 30 couples from the community (C), 25 couples with maladjusted children (M), and 22 couples who have schizophrenic offspring (S). Each of these parent couples had two or more children between the ages of 13 and 27. Additional S parents will be tested in the following year.

The M and S pairs did not differ appreciably in levels of general conflict, but reported more conflict than C couples. Although broad dominance variations were not discovered, certain item responses suggested that S wives might be more likely than other women to yield to their husbands in argument. Patterns of child conflict varied distinctly among the three groups, with M pairs reporting the highest amount, and S couples acknowledging slightly less conflict involving their non-schizophrenic children than C pairs. The schizophrenic index was involved in significantly more parental dissension than his next-of-age sibling. Parent-child alliances varied in terms of sex and indexicity among the diagnostic groups, with S mothers reporting considerable support from their daughters and little from schizophrenic sons.

Weighted responses to test items enabled accurate predictions of the diagnostic group to which each subject belonged, provided sex were known in advance (discriminant function analysis).

Serial No. M-AP(C)-14-10
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Evaluation of Family Dynamics with Conjoint Family Art Procedures.

Previous Serial Number: M-AP(C)-15-3

Principal Investigator: Hanna Yaxa Kwiatkowska

Other Investigators: None

Cooperating Units: Section on Adolescence and The Family; Unit on Longitudinal Studies

Man Years:

Total: 0.56
Professional: 0.36
Others: 0.2

Project Description:

Objectives: The purpose of the project is: (1) To evaluate families through a technique in which art media are used as a mode of communication and self-expression in order to compare (a) different families in which one or more of the offspring is afflicted with one of a variety of mental illnesses (schizophrenic, psychoneurotic, or delinquent) as well as normal families, and (b) different members within a family; (2) to evaluate through the same technique changes occurring in the course of family psychotherapy by repeating these procedures in periodic follow-ups during the treatment and after discharge.

Methods Employed: All members of a family are seen jointly in art evaluation sessions. The family is introduced to easy art media with no emphasis on artistic achievement. The structure of the session combines spontaneous self-expression with standardized procedures: (a) free picture; (b) family portraits; (c) abstract family portraits; (d) individual pictures started with the help of a scribble; (e) picture done by the family jointly and started with the help of a scribble. These procedures are devised for the comparison of different families. The sessions are conducted by the art therapist with a cooperating investigator, a psychiatrist or other staff member as participant observer. All the sessions are tape recorded, some of them video taped for a better study of family interaction. Abstracts of all the sessions, describing the families' transactions, their comments and discussions of their pictures as well as special observations of the investigators, are dictated upon completion of the session; the art productions and tapes of the sessions are studied for more accurate analysis. Each family is seen in a single evaluation session as close as possible after admission. Long term patients and their families are seen in a second family art evaluation after a minimum of six months of conjoint family therapy or at termination of treatment.

In view of the principal investigator's being officially connected with the project only through the first quarter of the fiscal year 1972-1973, work was concentrated on further analyses of the 1971-1972 findings on the investigated patient population. Observations on family dynamics of adolescents with behavioral problems, as seen in the Family Art Evaluation procedures, have been written up and are included as a chapter of the book "Family Art Therapy" commissioned by Charles C. Thomas, Publishers, Springfield, Illinois, to be published in the current year and authored by the principal investigator.

Major Findings: The specific features observed in these families were as follows: A great deal of verbal and pictorial material was produced, but while the pictures were impressively revealing of the families' dynamics, the investigators were amazed at the difficulty these families had in recognizing the problems and interrelationships so vividly displayed. The quantity of material by itself overwhelmed the investigators, but even careful study of tapes rarely revealed a deeper engagement of the family.

In the joint endeavor (see Method (e) above), the fathers made ineffective efforts at leadership and were frequently sabotaged by the mothers. The joint production was usually of an inferior quality than the family members' individual ones.

The separation issue was repeatedly revealed in the pictures of the families of this same group of adolescents. These adolescents underlined in their family portraits their desire for complete separateness from the family unit, but upon closer investigation one discovers that there is an ambiguity in these representations: the adolescent pictures himself either outside of the carefully delimited world of the family, yet somehow still closely attached to it or in complete unreality such as hazy, drugged fantasy world or on a desert island.

On the other hand, the opposite tendency was noticed in the parents. The myth of happiness and unity of the family was predominant, with only hints at scapegoating the index patient. Their pictures underlined the unity of the family and encircled tightly the family world.

Significance to Mental Health Research: The development of this technique continues to give additional dynamic understanding of family relations to projects of the Adult Psychiatry Branch of NIMH. The hypotheses based on the empirical findings obtained through family art evaluations have been scientifically investigated by systematic computer analysis of the material and described as a separate project, (M-AP(C)-14-12).

The researchers' and clinicians' interest in this technique continues to increase in the United States and abroad. As a result of the development of our work at NIMH, a graduate course in Family Art Techniques leading to a Master's Degree is offered by the Graduate School of Arts and Sciences at George Washington University and at Washington School of Psychiatry and is taught by the principal investigator.

Seminars on family art techniques, in the U.S.A. and abroad, reprints of papers, and consultations continue to be requested.

Honors and Awards:

The principal investigator has presented, by invitation, numerous papers and conducted workshops on family art techniques. Among others: the Annual Meeting of the District of Columbia Psychological Association; The University of Zagreb, Yugoslavia; Graduate School of Social Work and Community Planning, University of Maryland, Baltimore.

Publications:

Hanna Yaxa Kwiatkowska, Discussion of "Art Therapy - a Diagnostic and Therapeutic Tool" by Fink, Levick and Goldman, International Journal of Psychiatry, in press.

Hanna Yaxa Kwiatkowska, Requested review of book, "Art as Therapy with Children" by Edith Kramer, Journal of Nervous and Mental Disease, in press.

Serial No. M-AP(C)-14-11
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Family Art Therapy

Previous Serial Number: M-AP(C)-15-5

Principal Investigator: Hanna Yaxa Kwiatkowska

Other Investigators: None

Man Years:

Total: 0
Professional: 0 (inactive)
Other: 0

Project Description:

Objectives: (a) To further investigate the pilot study where family art therapy is used as the sole therapy with families of adolescents from different diagnostic groups; (b) To use this approach with families of different socioeconomic backgrounds.

Methods Employed: All members of a nuclear family are seen jointly in family art psychotherapy by the art therapist and the collaborating psychiatrist. Art media are offered to the family as a means of communication and self-expression; artistic or aesthetic considerations are not emphasized. In some of the sessions (at the beginning of treatment and repeated every six months) art evaluation procedures are used. These procedures combine free expression with standardized tasks and their purpose is to gauge the course of therapy periodically. Otherwise the family is largely left to structure the session; they are encouraged to draw or paint whatever comes to mind. All sessions are tape recorded; abstracts describing the family's interaction and the analysis of the pictorial and verbal material, as well as the therapists' subjective reactions, are dictated by both therapists. In the course of therapy at least one session is video taped.

Major Findings: No family was investigated this year, but a study based on a "blind" analysis by eminent psychiatrists and analysts of pictorial material obtained from long term family art psychotherapy and chronologically arranged was completed and presented by invitation at the American Psychiatric Association meetings under the title "Blind Evaluation of Pictures in Family

Psychotherapy: Their Communicative Power." This study demonstrated:

1. The blind judgements drawn from pictures only, without any verbal content were astonishingly accurate as to identity of index, diagnosis course, and outcome of therapy.

2. The observations of the judges brought more clarity on the reasons for a successful achievement of the therapy and helped the therapists gain a more precise understanding of their experience with this family.

Proposed Course: To complete for publication the monograph "The Use of Drawing and Painting as a Primary Mode of Communication in the Family Therapy of Schizophrenia."

Honors and Awards: An exhibit, "Pictures Help a Regressed Schizophrenic Patient to be Heard and Understood" by Hanna Yaxa Kwiatkowska, was presented by invitation at the Exhibition of Psychopathological Art at the Fifth World Congress of Psychiatry in Mexico City.

Publications: None.

Serial No. M-AP(C)-14-12
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Systematic Analysis of Family Art Evaluations

Previous Serial Number: M-AP(C)-15-8

Principal Investigators: James K. Dent, Ph.D. and Hanna Yaxa Kwiatkowska

Other Investigators: None

Cooperating Units: Section on Personality Development, Section on Twin and Sibling Studies, Section on Clinical Psychology, Division of Computer Research and Technology.

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Objectives: The overall objective is to explore systematically the materials available from the Family Art Evaluations which have been introduced by Mrs. Hanna Kwiatkowska and conducted for several years. Such an analysis is aimed at improving diagnostic procedures and increasing our understanding of the social psychological dynamics of psychopathology. In presenting the specific objectives, the longer range of objects are listed first; the immediate objectives are listed last.

A. Family Dynamics and Psychopathology.

1. To differentiate, through the characteristics of their art productions, families in which the index is diagnosed as process schizophrenic, reactive schizophrenic, psychoneurotic, and so forth.

2. To distinguish role positions in the family (father, mother, sibling, etc.), as revealed by their pictures (such as family portraits) and transactions.

B. Diagnostics (A Manual for the use of the Family Art Evaluations).

1. To specify the relation of the diagnosis of the index to the characteristics of his own art productions and those of his family.

2. To determine which characteristics of the art productions appear to represent transient symptoms and which represent more enduring personality characteristics.

C. Measurement Objectives.

1. To develop objective rating systems for pictures and family transactions in the Family Art Evaluation method.

2. To determine the interrater agreement and the internal consistency of the scales used.

3. To construct by combining scales, variables having stronger metric power.

4. To analyze the significance of variables like artistic talent and intelligence which may confound the relation between diagnostic variables and characteristics of the art productions. It is important to separate the evidence of psychopathology in a picture from the inference of intelligence or artistic talent.

Methods Employed: Our methods were described in detail last year. In summary, the pictures are blinded, randomized, and rated; one picture in four is check rated for studies of interrater agreement. A variety of statistical techniques are being used, depending upon specific objectives.

"Blind" ratings of pictures represent an independent measure of the mental status of an individual at a particular point in time. It is our plan to correlate these ratings with other data. Correlation with diagnosis and family characteristics such as socio-economic status will be greatly facilitated because some of these data have already been compiled in connection with the research done by Dr. Lyman C. Wynne.

Major Findings: Factor analysis across pictures reveals that there are some expected dimensions which are prominent and stable. For example, there is a dimension that might be labelled "bizarreness" which is always first or second in explaining variance regardless of how the factor analysis is conducted. However, there are many more independent dimensions than was anticipated; that is, even though interrater agreement is high, relations among dimensions tend to be low. The characteristics of the pictures are not easily summarized in a few dimensions.

Factor analysis across individuals indicated that there are a few dimensions which might be labelled "trait" dimensions. They extend across the various procedures, across two F.A.E.'s, and are correlated with intelligence and artistic talent. An example of such a dimension is the use of color. However, unexpectedly, "bizarreness" is not such a dimension; that is, an individual who produces a bizarre "first free" picture is not likely to show "bizarreness" in his last picture.

There are a large number of dimensions which show significant differences between patients and siblings. These differences are almost always as one would predict; for example, patients' pictures are more likely to be "bizarre." Comparisons between offspring and their parents are difficult because the parents tend to show less artistic talent. Further analyses of talent-free dimensions are required.

Significance to Mental Health Research: Since the art production is an independent, enduring representation of the mental status of an individual (or family) at a moment in time and in a structured family setting, it is a valuable datum both for enhancing our diagnostic methods and for investigating family dynamics in relation to mental disorder.

The usefulness of "drawing tests" for individual diagnosis is widely recognized. Considerable research has been done on the Draw-A-Person test. More recently there have been efforts to use other types of art productions. Although the literature is full of ideas about how to diagnose mental illness from pictures, most of the research has been concerned with a much simpler and less useful task, viz.: To predict whether a person is mentally ill. Only a few systematic studies (e.g., that of Dr. Robert Kaye) have been concerned with trying to specify what aspects of a picture are predictive of what kinds of mental disorder, and there have been no statistical studies of family dynamics as seen through the art productions of the whole family.

On the side of family dynamics, there is a rich body of research findings. Many of the concepts in this literature are used in the present study. Moreover, the material is being coded for the computer in the same format as for other studies in the Adult Psychiatry Branch, and this will greatly facilitate the comparison and integration of findings.

Proposed Course: Further Analyses of diagnostic groups will be conducted.

Honors and Awards: None.

Publications: None.

Serial No. M-AP(C)-14-13
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Systematic Analysis of Brazilian Family Art Evaluations: A Replication.

Previous Serial Number: M-AP(C)-15-9

Principal Investigators: Hanna Yaxa Kwiatkowska and James K. Dent, Ph.D.

Other Investigators: Dr. Carlos Paes de Barros, Dr. Aroldo Rodriques, Vera Pollo Flores, Luiz Duprat.

Cooperating Units: Institute of Psychology of the Catholic University in Rio de Janiero, Brazil, Department of State Committee on International Exchange of Persons (Fulbright Commission).

Man Years:

Total:	0.01
Professional:	0.01
Others:	0.00

Project Description:

Objectives: The overall objective is to explore the differences and similarities appearing in the systematic analysis of pictorial material obtained from Brazilian families with the material obtained from American families, here, in the identically structured situation of the "Family Art Evaluation." The specific objectives are:

1. To investigate and compare the characteristics of pictures drawn by Brazilian families where a family member has a specific psychiatric diagnosis with the characteristics of pictures of diagnostically similar families in the United States.

2. To define if eventual differences observed through this analysis could be attributed to cultural variations.

3. To study the differences in roles of family members in both countries as seen in the different procedures of the family art evaluations. E.G., in her work with Brazilian families Mrs. Kwiatkowska has observed that the family portraits of Brazilian families frequently include the extended

family, thus bringing to light the roles of substitute parents. Is there a correlation between the recurrent inclusion of such substitute parent in family portraits and the degree of pathology in the family? What is the role of the natural parents in such families?

4. To investigate and compare the interrater agreement on the same items of the rating system in the United States and Brazil. This could also shed light on cultural differences.

Methods Employed: The manual used for the NIMH study was translated into Portuguese by the Staff of the Institute of Psychology at the Catholic University in Rio de Janeiro. It was edited by Hanna Yaxa Kwiatkowska; staff members were trained by her personally to conduct a study parallel to the NIMH study.

A psychologist, Mrs. Vera Pollo Flores, with the consultantship of Miss Sylvia Beatriz Machado Joffely, is in charge of the study and under the direction of Dr. Carlos Paes De Barros, Chairman of the Department of Psychology. Other staff members of the Institute were designated as coordinators and trainers of the raters. The ratings were done by students as part of their regular curriculum for credit. Dr. Aroldo Rodriques, Research Psychologist, is a consultant for the statistical aspect of the study.

The patients referred to the Clinic at the Institute of Psychology are mostly children or adolescents in their early teens. Jointly with their families they all have a family art evaluation as part of their routine psychological work-up. A considerable amount of material has been accumulated since the evaluations were introduced there in 1966. In selecting sample families for the present study we were, nevertheless, limited by the age group; we do not want to include families where the index would be less than ten years old. Diagnostically the sample includes families of severely neurotic or behavior problem children, all out-patients at the Clinic. Records of all investigated Brazilian families were obtained and translated from Portuguese into English in order to obtain data (symptoms, onset, premorbid history) to be used in establishing diagnoses of the Brazilian subjects.

Major Findings: Factor analyses reveal that there are some similarities with and some differences from the factor structure of pictures drawn by our families here in NIMH. The clarification of omissions and discrepancies in the Brazilian data was helped by the assistance of Mrs. Junia Novaes, guest worker and graduate student from Brazil. It was possible to fill in most of the gaps in communication which were necessary to finish statistical analyses.

Significance to Mental Health Research: Family art evaluations provide a situation which offers a good deal of stability in the manner material is gathered for comparative studies. The basic material (pictures) is collected in an experimental setting structured as identically as possible with the NIMH setting. The methods used for the computer analysis of these data are also identical. The possible flaws in the translation and interpretation of the scoring manual, whether of linguistic or cultural nature are being investigated in a separate study (M-AP(C)-14-14 "Sources of Variance in Cross-Cultural Application of an Objective System for Analyses of Pictures Drawn by Patients and their Families").

Proposed Course: These analyses will be completed and written up during the current fiscal year.

Honors and Awards: None.

Publications: None.

Serial No. M-AP(C)-14-14
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Sources of Variance in the Cross-Cultural Application of an Objective System for Analyses of Pictures Drawn by Patients and Their Families.

Previous Serial Number: M-AP(C)-15-10

Principal Investigators: James K. Dent, Ph.D. and Hanna Yaxa Kwiatkowska

Other Investigators: Dr. Carlos Paes de Barros, Dr. Aroldo Rodrigues, Vera Pollo Flores.

Cooperating Units: Institute of Psychology of the Catholic University of Rio de Janeiro, Brazil.

Man Years:

Total:	0.01
Professional:	0.01
Others:	0.00

Objectives: A system has been developed for objectively classifying pictures drawn by patients and by members of their families in the Family Art Evaluations at NIMH (M-AP(C)-14-12). This rating system which consists of about fifty dimensions has been translated into Portuguese and is being used to describe pictures drawn by mental patients and their families in Brazil, (M-AP(C)-14-13). In application, the two measurements may not be equivalent for several reasons:

(a) Various pictorial symbols may have different significances in culture A (American) from those in culture B (Brazilian).

(b) The English and Portuguese translations may not be equivalent.

(c) Research group A may have particular frames of references not common to their own culture.

(d) Research Group B may also have particular frames of references.

The objective of this project is to begin to sort out some of these various sources of error and variance.

Methods Employed: Sixty-eight pictures selected at random from about one thousand pictures (American families) have been or are being rated as follows:

Ratings 1 and 2:

Two members of research group A have independently rated each of the pictures using the English translation.

Rating 3:

These raters discussed their differences and prepared a check rating representing the best judgment of research group A for each picture.

Rating 4:

Using the English translation, the pictures have been rated by two American college student volunteers working alone (no contact with the research group), each doing about half of the pictures.

Rating 5:

Using the Portuguese translation, a bilingual American college student volunteer rated the pictures alone (no contact with the research group).

Ratings 6, 7, and 8:

These are like ratings 1, 2, and 3 but are being done by Brazilian members of research group B in Brazil, from slides of the same pictures rated at NIMH.

For the 50 dimensions in the rating system, comparison among these ratings should permit us to isolate translation difficulties and some of the group effects. Presumably differences not attributable to these two sources of variance represent cultural factors in interpretation or individual rate biases. (A study of individual rater biases is included in M-AP(C)-15-8, 1970-1971). Because of the small number of pictures and of having only one bilingual rater, the results will have to be considered tentative. This project is essentially a pilot study for this type of investigation.

Major Findings: The Brazilians were able to rate only about half of the pictures we sent them. Analysis of this half indicated clearly which dimensions have a constant meaning in both centers, which have different meanings but are reliable within centers (group frames of reference), and which are so poorly defined they have little meaning in either center. Mrs. Junia Novaes, guest worker and graduate student from Brazil, has studied the various dimensions and has concluded that the differences between the Brazilian ratings and the NIMH ratings cannot be attributed to a lack of equivalence between the Portuguese and English versions of the rating system.

Proposed Course: Findings will be written up in the next fiscal year.

Significance to Mental Health Research: The precise description of mental disorders across cultures is hampered by a paucity of culture-free methods of assessment. Frequently the problem is dealt with by using the inferential judgments of particular clinicians who evaluate patients in two or more cultures. Agreement among them about particular patients is interpreted as constancy in measurement. This system which is biased by language, dialect and status problems may not provide replicable descriptions; i.e. there are no assurances that another group of clinicians with differing backgrounds would provide the same description, or even a common one.

At the other extreme, one is impressed by the almost universal understanding and appreciation of works of art. The messages of enduring representations of art seem on the one hand to be an expression of the different cultures and on the other hand to convey emotions universally accessible. One is also impressed with the high quality of communication in pure science. Where ideas are precisely defined in observable terms cross-cultural communication is facilitated. For these reasons objective descriptions of art productions may be an important way of achieving cross-cultural communication in description of mental disorders. In addition to being tangible the communication is not limited and/or biased by the language or dialect problem and the professional has a direct access to the experience of the patient.

Honors and Awards: None.

Publications: None.

Serial No. M-AP(C)-14-15
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Intellectual Factors in Mental Disorders Among Adolescents

Previous Serial Number: None

Principal Investigator: James K. Dent, Ph.D.

Other Investigators: None

Cooperating Units: Section on Clinical Psychology

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Part A:

Objective: To relate WAIS IQ and subtest patterns to diagnosis of mental disorders among adolescents.

Methods Employed: Data were obtained from the files of the Section on Clinical Psychology and are being analyzed by means of several multivariate techniques.

Major Findings: Preliminary findings indicate some consistencies with studies that are reported in the literature. Further analyses are needed to confirm these relationships.

Significance to Mental Health Research: Efforts to find WAIS patterns for various diagnostic groups have a long and somewhat discouraging history. It is generally believed that a major problem is the unreliability of diagnoses. The records, research and clinical, of the Adult Psychiatry Branch are a rich source of diagnostic material. For a number of years, in connection with this project and also 14-12, the investigator extracted information to form relatively homogeneous symptom patterns. The result is a large number of groups with small numbers in each. However, this permits comparisons with other studies which have similarly defined narrow diagnostic groups. Preliminary findings are encouraging. Discovery of such patterns would (1) provide a relatively objective aid to diagnosis, and (2) shed some light on intellectual factors associated with various disorders.

Proposed Course: To continue analysis, and to continue plodding through a large number of previous studies that have been done in this area. It is expected that these findings will be written up during the next fiscal year.

Honors and Awards: None

Publications: None

Part B

Objective: To relate WAIS IQ and subtest patterns to family role and mental disorders among adolescents.

Methods Employed: Same as for Part A above.

Major Findings: The conceptualization of intellectual factors in mental disorders appears to shift radically when one passes from the individual to the family constellation. Although there is evidence of intellectual factors (positive intercorrelations) within families, still the important findings probably surround the constellations of factors rather than these homogeneities. All present analyses are quite preliminary.

Significance to Mental Health Research: There are probably very few research sites that have IQ measurements for whole families. Some work has been done on the IQ's of siblings of schizophrenics. But otherwise, practically nothing is known. In fact, a major problem has been to search out whether certain observed patterns for these families are also found among normals (e.g., the correlation between parents' IQ).

Proposed Course: To continue the various multivariate analyses and conduct further searches of literature. It is doubtful that the investigator will have time to complete this work in the coming fiscal year.

Honors and Awards: None

Publications: None

Serial No. M-AP(C)-14-16
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Intellectual Factors in Unipolar and Bipolar Depressives

Previous Serial Number: None

Principal Investigator: James K. Dent, Ph.D.

Other Investigators: Dennis Murphy, M.D., Edward Donnelly, Ph.D.

Cooperating Units: Laboratory of Clinical Science

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Objective: To relate WAIS IQ and subtest patterns to diagnosis of unipolar and bipolar depressions. This project is similar to Part A of 14-15 except that these patients are adults. For these subjects there is a large volume of other data that may be useful in understanding the patterns which are found. For example, performance on WAIS subtests is known to be related to certain measures of trait anxiety, and of state anxiety, in a complex interaction. For these patients, MMPI profiles are available, and also observational data. The role of anxiety in these affective disorders is of considerable interest.

Methods Employed: Multivariate analysis of test data.

Major Findings: Substantial WAIS patterns are found for the two groups. The meaning of these patterns is problematic until further analysis has been conducted.

Significance: See Objectives above.

Proposed Course: To continue the analyses and write up the findings, hopefully in the coming fiscal year.

Honors and Awards: None

Publications: None

Serial No. M-AP(C)-15-2
1. Adult Psychiatry Branch
2. Family Studies Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Study of the Separation Process in Adolescents and Their Families

Previous Serial Number: M-AP(C)-14-8

Principal Investigator: Helm Stierlin, M.D., Ph.D.

Other Investigators: Stanley I. Hirsch, Richard M. MacDonald, M.D., Robert J. Savard, Ph.D., Margaret T. Singer, Ph.D.

Cooperating Units: Section on Clinical Psychology; Section on Perceptual and Cognitive Studies; Section on Psychiatric Assessment (Adult Psychiatry Branch, NIMH).

Man Years:

Total:	3.0
Professional:	2.2
Others:	.8

Project Description:

Objectives:

The basic objectives of this study have remained the same as stated in the report of last year: to investigate, at the clinical and research levels, the individual and family factors which may further or jeopardize the separation process throughout adolescence. By tapping a large available reservoir of students with school problems, the study makes use of an adolescent high risk population who, over the following five years, will or will not develop either in a psychotic, neurotic or sociopathic direction. Understanding and possibly differentiating the separation course of these three patient groups is the main goal. This includes a grasp of these patients' family and peer relations as they unfold in time. The project builds on many of the findings which Drs. Lyman C. Wynne and Margaret T. Singer and their associates have reported during the last decade. It addresses itself now to experimental factors which may allow certain high risk adolescents to steer clear of serious psychopathology while causing others, of seemingly equal or even lower risk, to succumb to such psychopathology.

Within the framework of this broad objective, the study tries, further, to throw light on several specific related issues. These are:

(a) Parental perceptions and expectations which specifically bear on their children's separation, e.g., their ability and willingness to seek partners outside the family or to take on responsible jobs. We have become increasingly impressed with the importance of such perceptions as factors which either inhibit or promote their children's moves toward autonomy.

(b) The individual and family dynamics of runaway adolescents. Twenty-six out of 43 index adolescents seen in this project had run away from home at least one full day and night, before they had turned 17 (some of them have run away frequently, getting as far away as Florida, California, and Canada). The project offers a unique chance to study in depth this group of runaways.

(c) The often divergent developmental course of siblings who appear close in age and who at first sight appear exposed to similar family influences.

(d) The sequela of an early precocious and overstimulated development.

(e) Psychological and perceptual aspects of teenage drug abuse.

(f) The potential problems (particularly those relating to countertransference) and limits inherent in various treatment approaches - but mainly individual, couple, and family therapy - as used during a crucial juncture in an adolescent's life and separation process.

(g) The interlocking life crises of middle-aged parents and adolescent children.

(h) The role of the peer group in facilitating or hindering the adolescent's growth and separation.

(i) The similarities and differences between family myths and group fantasies.

(j) The roles of shame and guilt in adolescent separation.

(k) In collaboration with Dr. Margaret Singer, the Rorschach protocols of our families are being systematically studied. The main goal here is to transform the clinically derived concepts of transactional modes (i.e., the modes of binding, delegating, and expelling) into instruments for the evaluation and prediction of crucial adolescent separation dynamics.

Methods Employed:

Adolescents of age 15 and 16 who attend public schools in Montgomery County (Maryland) supplied the bulk of our patient material, in addition to those referred to us by private psychiatrists and various social and psychiatric agencies. The various referral sources sent us adolescents who demonstrated school problems and whose families appeared willing to engage in family therapy. It was assumed that these students represented a high risk population both in the direction of schizophrenia and in the direction of sociopathic development. Mostly unbroken families were accepted. At the initial screening appointment, the family was seen as a group for 50 minutes and then the couple and designated patient were seen each for one-half hour. During the first two years of the project, families were admitted only to outpatient therapy of at least three months duration. From the third year on, we began to accept inpatients also. During the fiscal years 1970 through 1972, we shifted exclusively to an inpatient program for the index adolescents which was gradually phased out during fiscal year 1973. Within the inpatient program, the patient was admitted to Unit 3-West where he received individual psychotherapy, usually three times a week. The family was seen in once- or twice-weekly family therapy sessions. Couple therapy once a week became mandatory. Individual psychotherapy for family members was offered when necessary and feasible. On the ward, the intensive therapy of the individual adolescent was supplemented by an intensive milieu program. No time limits were set to the individual or family treatment except for those dictated by the lengths of the therapists' stay at NIMH.

After the first screening interview the available historical, social, and dynamic data about the family were summarized and a fairly detailed prediction was made about the index patient. This prediction included ten major items such as employment in five years, psychiatric contacts and diagnoses, suicidal tendencies, delinquent tendencies, etc. The ensuing family and individual therapy was then used to obtain a more comprehensive picture of the family dynamics as they bore on the adolescent's separation problem and further development. The following areas were carefully observed and assessed:

(1) The prevailing parental perceptions and expectations, particularly insofar as these bear on the adolescent's separation.

(2) All data pertaining to runaway episodes of adolescents, e.g., overt or covert parental encouragement, the context of the running away episode(s), antecedents, peer contacts, etc.

(3) The communication patterns within the family. We tried to establish how much each family was bound to sabotage a pooling of resources.

(4) The nature of the family's alignments, their relative shifts and inconsistencies, their strengths, and their overt- or covertness.

(5) Family boundaries. We tried to assess how the family is integrated within the community and how much it might put obstacles in the way of the adolescent associating with peers, teachers, and other figures outside the family.

(6) Patterns of psychological exploitation: The various ways in which a child (more or less covertly) was recruited to serve his parents in ways which interfered with his own growth and separation.

(7) We tried to be alert to all data, implicit and explicit, which could throw light on the parents' roles in the adolescent's separation and, in particular, on their own separation problems when they were adolescents themselves.

(8) Through an actively and empathically conducted family therapy, we tried to get an indication of how much the family might be capable of changing its

patterns and thereby facilitate the adolescent's separation. Also, by focusing on the evolving transferences and countertransferences, we tried to get further clues about the parents' own separation problems.

On the basis of these assessments, which were supplemented by an individual interview with each family member, we made, at the end of the treatment period, second step predictions about the adolescent's prospective life style at the age of 21. These predictions covered the same items as those made immediately after the screening session, but they were now amplified. Also, we supplemented them by a narrative kind of prediction which dealt with the question of how much the index would become restricted or nonrestricted, active or more passive. In cases where there were two siblings closely related in age, the two of them were covered by predictions. It was expected that the latter confirmation, partial confirmation, or refutation of our predictions would deepen our insights and sharpen our hypotheses about the adolescent separation process. The comparison between the first step and second step predictions, as outlined above, appeared as a useful means of alerting us to possible sources of predictive error at this early stage of the research project.

While the principal clinical investigator tried to make five-year predictions based on the assessment of presumably crucial and lasting family features, as mentioned earlier, additionally obtained Rorschach tests, TAT, cognitive and perceptual style procedures of all family members, as well as the audio and video tapes, provided material for independent evaluations and predictions to be made either then or at later stages of the project. The availability of these test procedures made it further possible to utilize these families for other ongoing research projects of the Adult Psychiatry Branch, as described elsewhere.

Further, a standardized diagnostic inventory was made in accord with guidelines developed by Drs. John Strauss and Will Carpenter on all newly admitted index patients. This should provide us with a further means to check out (subjectively tinged) dynamic family data against other independently obtained and more standardized data.

Altogether 43 index patients and their families have been included in the program. An additional 19 families were seen in screening sessions only. These families, too, were made subject to predictions so that they could be followed up and could serve as untreated controls to the treated index patients and their families.

No new families were admitted during the last year and those still in treatment at the beginning of the current fiscal year are being phased out. (At present, only one of a total of 43 families is still being seen as outpatients.) The major focus has continued to be the final follow-up interviews carried out by Drs. Stierlin and Savard. These follow-ups are by now almost completed, as only three more families will be seen. Seven families either refused to be interviewed or moved so far away as to make systematic follow-up impossible. Yet also on all these "refractory" families we obtained at least some information. Most of the follow-up interviews were conducted in the families' homes.

In these interviews, index patients, siblings close in age, and the two parents were seen for approximately 50 minutes each. The assessments directed to them covered the areas of earlier made predictions. Following these individual follow-up interviews, all family members were asked to listen to excerpts from audio or (when available) video-tapes made at earlier conjoint family therapy sessions and dating back up to five years. The family members were then encouraged to reflect together on how the situation conveyed by the tape excerpt did still exist and on how they all had changed or not changed. In the ensuing discussion there emerged often items and issues which had not been covered in the preceding individual follow-up interviews.

Major Findings:

The systematic evaluation of the follow-up interviews is now under way. The following findings have already been noted and either published or are ready for publication.

1. The two major patterns in adolescence, called centripetal and centrifugal, which were mentioned in previous reports, have been further refined. With an ongoing focus on these differing separation patterns, the conceptual framework which emerged during the last two years was further developed. Crucial to the framework is the concept of transactional parental modes which all, albeit in different ways, contribute to a distorted separation of adolescent children. They operate via parental perceptions and expectations but also imply more basic and mutual relational ways and styles.

2. In line with the above, differing separation conflicts of adolescent children could be further delineated. The differing implications for the parent's and adolescent's growth and the resulting differing indications for therapeutic intervention in separation conflicts have been spelled out.

3. The role of parental perceptions and expectations for the development of crucial identificatory processes in the adolescent has been further clarified. In particular, certain interpersonal factors bearing on varying forms of internalizations (which include incorporations, introjections, and identifications) could be systematically described.

4. Further work was done on the family dynamics of runaways which were mentioned in last year's report.

5. The similarities and differences between group dynamics and family myths were clarified.

6. Major family and separation dynamics in schizophrenia and their treatment implications were described.

7. Within the emerging complex conceptual framework, a new perspective on countertransference in the family therapy with adolescents was developed.

8. Preliminary criteria for the application of the clinically derived concepts of transactional modes (binding, delegating, expelling) to the evaluation of Rorschach and TAT protocols were established.

Significance to Mental Health Research:

This study builds on findings about the structures, communication patterns, and boundaries of families with disturbed offspring which have been made in the Adult Psychiatry Branch during the past decade. With the focus on longitudinal development and the separation process in adolescence, this study tries to assess the significance of various - mainly experiential - factors in the actual outbreak of schizophrenia and other disturbances during late adolescence, a period which is known to be fateful in an individual's personality development and which yields a high percentage of first schizophrenic breaks. The new focus on runaways appears commensurate with the increasing social importance of this patient group.

In the present study, family therapy is used as a diagnostic instrument. At the same time, it is tried out as a possibly efficient and most economic tool in the prevention of the major disasters of late adolescence.

Proposed Course of Project:

The follow-up interviews on the approximately forty families who have passed through this project have been nearly completed, but it has become evident that another year will be needed to make good use of the rich data which this project has yielded.

Honors and Awards:

By invitation, participated in the Psychotherapy of Schizophrenia Conference, Austen Riggs Center, Inc., Stockbridge, Massachusetts, October 13-14, 1972.

By invitation, participated in the Symposium on Family and Social Dynamics in Schizophrenia, Department of Behavioral Sciences, Eastern Pennsylvania Psychiatric Institute, Philadelphia, Pennsylvania, April 12-13, 1973.

Publications:

Stierlin, H.: Family dynamics and separation patterns of potential schizophrenics. In Alanen, Y. O. (Ed.): Proceedings of the Fourth International Symposium on Psychotherapy of Schizophrenia. Amsterdam, Excerpta Medica, 1972, pp. 169-179.

Stierlin, H. and Ravenscroft, K., Jr.: Varieties of adolescent "separation conflicts." Brit. J. Med. Psychol. 45: 299-313, 1972.

Silverman, J., Buchsbaum, M., and Stierlin, H.: Sex differences in perceptual differentiation and stimulus intensity control. In press, Journal of Personality and Social Psychology.

Stierlin, H.: Countertransference in the family therapy with adolescents. In press, Sugar, M. (Ed.): The Adolescent in Group and Family Therapy.

Stierlin, H.: A family perspective on adolescent runaways. In press, Arch. Gen. Psychiat.

Stierlin, H.: Family therapy with adolescents and the process of intergenerational reconciliation. In press, Sugar, M. (Ed.): The Adolescent in Group and Family Therapy.

Stierlin, H.: Group fantasies and family myths. In press, Family Process.

Stierlin, H.: Psychoanalytic approaches to schizophrenia in the light of a family model. In press, Int. J. Psycho-Anal.

Stierlin, H., Levi, L. D., and Savard, R. J.: Centrifugal versus centripetal separation in adolescence. In press, Feinstein, S. and Giovacchini, P. (Eds.): Annals of the American Society for Adolescent Psychiatry, Vol. II: Developmental and Clinical Studies.

Serial No. M-AP(C)-15-6
1. Adult Psychiatry Branch
2. Family Studies Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Conceptualization of Lasting Dyadic Relationships

Previous Serial Number: M-AP(C)-14-9

Principal Investigator: Helm Stierlin, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.4
Professional:	.2
Others:	.2

Project Description:

Objectives:

This project seeks to contribute to the theoretical base for the comparative analysis of a wide variety of human relationships. It grew out of conceptualizations which the investigator developed before he joined the Adult Psychiatry Branch seven years ago and which were published in book form (Conflict and Reconciliation. New York: Doubleday-Anchor, 1969; Science House, 1969). The focus continues to be on the ongoing transaction between two persons who are considered as subjects - that is, as centers of an individual orientation toward the self, the world, and the other - and who are changed while the relationship unfolds. The phenomenology and dynamics of psychological exploitation appear here particularly significant. The implications and limits of a dyadic model for the better understanding of family and group relationships are being explored. Also, creativity as a response to a binding and conflictual interpersonal field has been made a special focus of study.

Methods Employed:

Study, critical evaluation, and integration of a wide range of ideas and findings reported in the literature of human relationships. At the same time, the project relies increasingly on the clinical experiences and observations provided through the clinical service of the Adult Psychiatry Branch and particularly the ongoing family therapies. Increasingly, the issues raised and described in Project M-AP(C)-15-2 have influenced the here-described conceptual efforts.

Major Findings:

(1) The concept of psychological exploitation has been further developed and clarified and has been integrated with the concept of transactional modes. Specifically, it was related to how needs, power, and interpersonal justice evolve and are defined in human relationships.

(2) The principal investigator has again taken up his interrupted work on Karl Jaspers, which resulted in a lengthy paper (see Publications).

Significance to Mental Health Research:

This conceptual work is relevant to the understanding of schizophrenia as a transactional thought disorder. Beyond that, it seeks to illuminate various mental disorders as being linked to special types of human relationships. The complexities of human relationships, it appears, have far outdistanced the conceptualizations so far at our disposal. This project tries to reduce the conceptual lag in this area. The concept of psychological exploitation seems relevant to many pressing social issues.

Proposed Course of Project:

The principal investigator is working on a book which pulls together the thoughts and themes developed under this project.

Honors and Awards: None

Publications:

Stierlin, H.: Lyrical creativity and schizophrenic psychosis as reflected in Friedrich Hölderlin's fate. In George, E. E. (Ed.): Friedrich Hölderlin/An Early Modern. Ann Arbor, University of Michigan Press, 1972.

Stierlin, H.: Hölderlins dichterisches Schaffen im Lichte seiner schizophrenen Psychose. Psyche 26: 530-548, 1972.

Stierlin, H.: Self-actualization and philosophical awareness. In Burton, A. (Ed.) and Associates: Twelve Therapists/How They Live and Actualize Themselves. San Francisco, Washington, London, Jossey-Bass, 1972.

Stierlin, H.: Das Tun des Einen ist das Tun des Anderen. Frankfurt, Suhrkamp Verlag, 1972.

Stierlin, H.: Karl Jaspers' psychiatry in the light of his basic philosophic position. In press, Journal of the History of Behavioral Sciences.

Serial No. M-AP(C)-16-1
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: WHO International Pilot Study of Schizophrenia

Previous Serial Number: M-AP(C)-16-1

Principal Investigators: William T. Carpenter, Jr., M.D.
John S. Strauss, M.D., Co-principal Investigator, University of Rochester School of Medicine and Dentistry

Collaborating Investigator: John Bartko, Ph.D.

Cooperating Units: Biometrics Branch, NIMH
University of Rochester Medical School
Prince Georges General Hospital
Spring Grove State Hospital
Morris Cafritz Memorial Hospital
World Health Organization Field Research Centers: Colombia, Czechoslovakia, Denmark, Great Britain, India, Nigeria, Taiwan, and Union of Soviet Socialist Republics

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: (1) To develop standard interview and rating schedules that can be used cross-culturally for the evaluation of psychotic patients. (2) To evaluate a cohort of patients from the catchment area of Prince Georges County, Maryland with these instruments and to compare these findings with similar evaluations being carried out by investigators in eight other psychiatric centers in Colombia, Czechoslovakia, Denmark, Great Britain, India, Nigeria, Taiwan and the USSR. (3) To work toward a consensus with investigators from these other countries about criteria derived from the interview instruments, for classifying patients as schizophrenic. (4) To compare these WHO diagnostic evaluations with clinical evaluations and ratings of the same patients made with other methods such as the Lorr IMPS scale and projective tests. (5) To compare social and demographic data on patients evaluated by the standard instruments to understand more completely how these factors are related to schizophrenia as defined through the use of the standard diagnostic schedules. (6) To compare course of illness in patients from the collaborating centers by conducting a follow-up study on patients seen in the original sample.

Methods Employed: Standardized forms have been developed for evaluating mental status, history, social function, and outcome. Initial evaluations were completed using these forms of 135 patients from each center. Data is being analyzed to compare the patients from the nine centers in terms of initial diagnosis, social function, and follow-up status. Alternative diagnostic techniques have been applied to evaluate their validity.

Major Findings: This year Volume One of the IPSS report has been published. This report described methods and findings of the first phases of the IPSS. These phases include (1) the preparation, translation, pretesting, and revision of the data collection instruments; (2) the application of these instruments to 1,202 patients in nine different centers; (3) the evaluation of reliability of the interview schedules; and (4) diagnostic comparisons of the patients seen in the nine centers.

The preparation and translation of data collection schedules of this kind with collaboration of psychiatrists from nine centers is, in itself, a significant contribution to the methodology available to future cross-cultural studies. In evaluating the applicability and reliability of the schedules, two findings were especially significant. First, it was possible to use the same standardized mental status interview (in translation where necessary) in all of the collaborating centers and reliability of ratings within each of the centers was high. The reliability of the ratings across centers was at a lower but still acceptable level. Because history and social description data had to be collected in different ways in the different centers, and because these data depend so much for their psychiatric significance on the cultural milieu, it was more difficult to evaluate the comparability of these kinds of information from center to center. However, the schedules provided a useful means of collecting these data and represent a first step towards the development of comparable methodology in this area as well.

The comparison of similarities and differences in patient types seen in the different centers has been given considerable attention. The inherent complexity of such a comparison, even of patients within one center, is often underestimated, but was highlighted by the cross-cultural nature of this project. Three different methods for defining diagnostic types were used. The results of these three different methods were then compared. In the first diagnostic method, the interviewing psychiatrist assigned the patient to one of the commonly used clinical diagnostic categories. The categories defined by the International Classification of Diseases were those most frequently used; however, some centers tended to use special diagnostic categories. Analysis of symptom profiles of the different diagnostic types showed broad similarities across centers for the general diagnostic categories such as schizophrenia, and manic-depressive psychosis. It indicated further that patients with some of the common defining characteristics of these diagnostic categories were seen in all centers. Comparison of symptom profiles to diagnostic labels applied by investigators from the centers also clarified many differences in the way in which psychiatrists from different centers used symptom information in reaching a diagnosis.

A second diagnostic method used to analyze the types of patients seen in this study was a computer program developed to simulate clinical diagnoses as they might be made by the psychiatric school led by Kurt Schneider. Using data from the mental status examination and psychiatric history, this computer method (Catego) was able to duplicate, to a large extent, the broad clinical diagnoses such as schizophrenia, and psychotic depression given to the patients by the investigators in the centers. This method represents considerable progress in developing a technique for applying diagnoses to patients which can be applied in different centers with complete reliability.

The third method used for assigning patients to diagnostic categories employed cluster techniques. With these mathematical techniques for defining groups of patients, it was possible to arrive at categorizations that were somewhat similar to both clinical and Catego diagnoses. The cluster techniques also provided a means for defining certain subgroups of patients that were seen in only one or two centers. The results of these three methods were compared and combined to describe basic similarities and differences among patients seen in the different centers.

The data from the study is also being used as source for other closely related investigations: (M-AP(C)-16-3, M-AP(C)-16-4). These studies involve more intensive investigation of certain aspects of the data obtained during the IPSS and comparison of these data with similar information obtained from other sources.

Significance to Mental Health Research and the Program of the Institute: The difficulty in comparing psychiatric patients from different cultures has made it impossible to evaluate accurately the etiological importance of cultural factors in psychiatric disorder. The absence of standard methods for evaluation and diagnosis of psychiatric patients has also made it difficult to compare incidence and prevalence rated in different cultures and to compare usefulness of treatment methods employed in different centers. Until these comparisons can be performed, it is not possible to test many crucial hypotheses regarding the nature and etiology of psychiatric disorders. The difficulties in establishing standard instruments are considerable, not only in the writing and testing of such instruments, but in the extensive negotiations and communications necessary with the various investigators from different countries with diverse backgrounds and orientations. Nevertheless, in this study it has been possible to develop such instruments that can be applied in a useful way in a variety of cultural settings. These instruments were used with patients in nine different countries and proved to be applicable and useful for this purpose.

While methods of evaluating patient characteristics must be developed in order to perform cross-cultural studies, it is also essential to develop methods for categorizing patients once their characteristics have been elicited and rated. In the current studies, three different methods were used to categorize patients and to compare patient types among the different centers. Through applying these methods, it is possible to show in what sense patients seen in the different centers are similar and in what sense they are unique. The combinations of these methods can also be used to determine in what sense the statement, "schizophrenia is found in all of the nine centers," is true, and in what sense the statement omits recognition of real differences of "schizophrenic" patients from the different centers.

The completion of a follow-up study on the patients originally interviewed adds the crucial dimension of course of illness to the evaluation. By having standardized, comparable information on patients from different countries at two points in time, it will be possible to compare course of illness cross-culturally and to investigate further the use of outcome of psychiatric illness to validate the original diagnoses. Data analysis of the two year follow-up has begun.

Proposed Course of the Project: In the coming year data analysis from the follow-up phase of the IPSS will be completed and preparation of Volume Two of the IPSS report will be undertaken reporting follow-up results. Eight of the countries will complete data collection for the five year follow-up. Uncertainty about future support has precluded a decision to date regarding the Section's (United States) participation in the five year follow-up.

Publications:

International Pilot Study of Schizophrenia, Volume I. World Health Organization, Geneva, Switzerland, 1973.

Serial No. M-AP(C)-16-2
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Pseudo-therapeutic Community

Previous Serial Number: None

Principal Investigators: Michael H. Sacks, M.D.
William T. Carpenter, Jr., M.D.

Man Years:

Total:	.2
Professional:	.18
Other:	.02

Project Description:

Objectives: To develop the concept of the pseudo-therapeutic community and contrast it to a therapeutic community. A pseudo-therapeutic community is defined as a psychiatric unit advocating a therapeutic belief system while covertly functioning in opposition to their definition of what is therapeutic.

Methods Employed: Therapeutic communities were observed. One community was closely examined utilizing a study group consisting of administrative and therapeutic personnel.

Major Findings: The tendency toward becoming a pseudo-therapeutic community is present in all psychiatric units. Five prominent group interactions characterize a pseudo-therapeutic community. They are: (1) the absence of a therapeutic standard; (2) assignment of irresponsibility; (3) establishment of an anti-therapy leader; (4) the absence of therapeutic leadership; and (5) maintaining a pathogenic environment (the community double bind).

Significance to Mental Health Research and the Program of the Institute: The processes and concepts of the therapeutic community have found wide acceptance and it is the prevalent therapeutic philosophy on most psychiatric units. There are, however, powerful interferences to the successful operation of a therapeutic community which may produce a pseudo-therapeutic community. Conceptualizing the pseudo-therapeutic community provides a framework for understanding the natural tendency toward operating in this mode and the interactions that characterize it. Increased sensitivity to these elements foster the capacity for psychiatric units to maintain their therapeutic efficacy.

Presentations:

The Pseudo-therapeutic Community, presented by Dr. Sacks to the Psychiatric Staff of the Washington Hospital Center, April, 1973 and to the Eighth Joint Meeting of the Professional Associations of the U. S. Public Health Service in Phoeniz, Arizona, May, 1973.

Serial No. M-AP(C)-16-3
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Comparative Studies of Functional Psychoses

Previous Serial Number: M-AP(C)-16-3

Principal Investigator: William T. Carpenter, Jr., M.D.

Collaborating Investigators: John S. Strauss, M.D.
John Bartko, Ph.D.

Cooperating Units: University of Rochester Medical School
Biometrics Branch
Prince Georges General Hospital
Spring Grove State Hospital
Morris Cafritz Memorial Hospital
World Health Organization
3-E and 4-W Nursing Units, Clinical Center, NIH
Section on Psychiatry
Laboratory of Clinical Science

Man Years:

Total:	0.4
Professional:	0.3
Others:	0.1

Project Description:

Objectives: (1) To utilize standard interview and rating schedules to collect data on manic-depressive patients. (2) To utilize this data and data collected on a larger group of schizophrenic patients in a comparable manner to investigate similarities and differences in symptomatology within these diagnostic categories. (3) To provide a sufficiently large sample of these manic-depressive patients to enable comparison with similarly diagnosed patients from other field centers in the WHO International Pilot Study of Schizophrenia. (4) To obtain data with which to test the hypothesis that many of the usually accepted criteria for a depressive illness (i.e., biological signs, psychosomatic complaints, sleep difficulties, decrease in libido, etc.) do not have high order discriminatory function between depression and other forms of mental disturbances. (5) To generate a sufficient sample of depressed patients to explore the hypothesis that a diagnosis of schizophrenia is often used for a person who otherwise is comparable to a psychotic depression but has a bizarre delusion or the presence of an hallucinatory phenomena which many clinicians in practice do not identify as part of a depressive illness. (6) To elucidate the concept of schizo-affective schizophrenia by examining the signs and symptoms present in cases so diagnosed comparing them with depressed patients and schizophrenic patients during illness and through a follow-up period. (7) To test empirically current diagnostic systems for the psychoses.

Methods Employed: The standard instruments were developed for the WHO International Pilot Study of Schizophrenia. They were developed, revised, and extensively pretested on at least 24 patients from centers in eight countries and have been used in the past year on a cohort of 135 patients from each center. Four basic interview forms comprise the standard instruments and are described in project No. M-AP(C)-16-1. Using these schedules on a sample of patients not included in the IPSS, 35 manic-depressive patients have been interviewed from wards 3-E and 4-W in the Clinical Center, NIMH. Computerized statistical methods are being developed to analyze the data collected from these groups of patients to develop empirical "clusters" of patients based on the symptoms they demonstrate. The material on certain sub-groups of patients (i.e., schizo-affective schizophrenia) will be examined by the principal investigators in an effort to clarify descriptive and conceptual aspects of the diagnostic categories. Analysis of variance and discriminate function techniques have been used to determine what signs and symptoms have highest discriminating value.

Major Findings: It has been possible to accumulate systematic symptom and sign data on large groups of patients assigned to various functional psychotic subcategories. This data permits testing of major diagnostic concepts and to contrast symptom profiles among various diagnostic categories. The most important finding to date comes from the first empiric investigation of Kurt Schneider's diagnostic concept. Schneider's identification of pathognomonic symptoms of schizophrenia has influenced diagnostic practices throughout most of the world. Despite general acceptance for over forty years, his diagnostic system has never been empirically tested. Using the patient data described above, we were able to demonstrate that the first rank symptoms are not pathognomonic for schizophrenia, but occur in 23% of manic-depressive patients and in 9% of neurotic and character disorder patients. We have been able to replicate these findings with the data from each of the other eight centers in the IPSS.

A group of highly discriminating symptoms for schizophrenia have been identified. A diagnostic system has been constructed using the presence of these symptoms to determine the degree of confidence in a schizophrenic diagnosis.

These data have been used with a number of diagnostic procedures to identify groups of patients to test hypotheses related to outcome in schizophrenia (see Project Serial No. M-AP(C)-16-7).

Significance to Mental Health Research and the Program of the Institute: There is a paucity of data in functional psychoses using standardized interview techniques. Evaluating diagnostic groups and comparing them with similar groupsfrom the various field centers in the WHO International Pilot Study of Schizophrenia offers an opportunity to clarify the use and misuse of the diagnostic categories and to describe more clearly phenomena represented in each and the distinguishing features of the various diagnostic categories. The ability to identify homogeneous and comparable groups in various centers is fundamental for all human research of the psychoses.

Proposed Course of the Project: During the coming year we will test other diagnostic concepts. We will use follow-up information and premorbid data in an effort to validate and invalidate diagnostic groups. Mathematical approaches to this data can help identify the critical symptom variables in diagnosing. Revision of diagnostic concepts will follow in order to maximize their ability to discriminate between patient groups based on signs and symptoms that occur frequently and are readily observed by clinicians.

Publications:

Carpenter, W. T., Jr., Strauss, J. S., and Muleh, S.: Are There Pathognomonic Symptoms in Schizophrenia?: An Empiric Investigation of Kurt Schneider's First Rank Symptoms. To be published in Arch. of Gen. Psychiat., 1973.

Presentations: Presented by Dr. William T. Carpenter, Jr.

1. The Search for Pathognomonicity in Schizophrenia, presented to the Eleventh Annual Conference of the Mental Health Career Development Program, New Orleans, Louisiana, 1972.

2. Pathognomonicity in Schizophrenia: An Empiric, Cross-Cultural Investigation of Kurt Schneider's Diagnostic Concept, presented at the Annual Meeting of the American Psychiatric Association, 1972.

3. On the Identification of Schizophrenia, presented to the Department of Psychiatry, State University of New York at Buffalo Medical Center, 1972.

4. The Continuing Search for Highly Discriminating Signs and Symptoms of Schizophrenia, presented to the Twelfth Annual Conference of the Mental Health Career Development Program, Williamsburg, Virginia, 1973.

5. A Proposal for the Identification of Schizophrenia: A Report from the International Pilot Study of Schizophrenia, presented to:
   a. The Department of Psychiatry, Westchester Division of Cornell University Medical College, 1973.
   b. The Department of Psychiatry, University of Rochester School of Medicine and Dentistry, 1973.

6. A Flexible System for Identifying Schizophrenia, presented in the New Research Reports, Annual Meeting of the American Psychiatric Association, May 1973.

Serial No. M-AP(C)-16-4
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Empirical Grouping of Psychiatric Patients

Previous Serial Number: M-AP(C)-16-4

Principal Investigators: William T. Carpenter, Jr., M.D.
John S. Strauss, M.D.
John Bartko, Ph.D.

Cooperating Units: Biometrics Branch, NIMH
Computer Center, NIH
World Health Organization

Man Years:
Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objectives: (1) To develop methods for grouping psychiatric patients empirically. (2) To determine whether patients as defined by their symptoms fall into natural groups or vary along continua. (3) To evaluate methods commonly used for the empirical grouping of patients.

Methods Employed: In the process of analyzing the data generated by the WHO IPSS (M-AP(C)-16-1), some difficulties with commonly used clustering and factoring procedures for grouping patients became apparent. The investigators have used a series of statistical techniques to locate where in the process of data analysis the difficulties arose and to understand the relationships between cluster techniques and the underlying discrete or continuous structure of data.

Major Findings: The results of the commonly used clustering and factoring techniques for grouping psychiatric patients have been demonstrated to be less stable than is generally recognized. It has been possible to identify the variables that are significant in causing this instability. The inability of even the improved techniques to create stable clusters from psychiatric data suggests that psychiatric patients may represent continua rather than discrete categories.

Significance to Mental Health Research and the Program of the Institute: Because of the unreliability of psychiatric diagnoses and their limited utility, many investigators have turned to mathematical models to define more meaningful groups of psychiatric patients. These efforts may one day be important in defining groups of patients with similar causes and courses of their disorders and similar responses to treatment and thus provide considerable progress in research, treatment, and prevention of psychiatric disorders. The mathematical models currently in use have many weaknesses that must be recognized and eliminated before such a step can take place. By comparing different methods of data analysis with the same group of patients, we have identified some of these weaknesses, attempted to develop a more useful technique of data analysis with more meaningful and stable output and to use this technique to study how appropriate typological diagnostic concepts are to the nature of psychiatric disorder.

Proposed Course of the Project: The work completed has been prepared for publication. Further efforts continue along two major directions: (1) the use of different techniques to define more of the problem areas and to develop more satisfactory methods of grouping patients; (2) an alternate direction, the developing of dimensions of psychiatric function for identifying psychiatric patients rather than attempting to place patients into discrete groups; (3) an investigation of schizophrenic subgroups.

Publications:

Bartko, J. J., Strauss, J. S., and Carpenter, W. T., Jr.: An Evaluation of Taxometric Techniques for Psychiatric Data. Classification Society Bulletin, 2(3), 1971.

Strauss, J. S., Bartko, J. J., and Carpenter, W. T., Jr.: The Use of Clustering Techniques for the Classification of Psychiatric Patients. To be published in the Brit. J. Psychiat., 1973.

Presentations:

Diagnostic Models and the Nature of Psychiatric Disorder. Invited paper presented at the Annual Meeting of the Classification Society, Chicago, April 24, 25, 1972, by Dr. John Strauss.

Serial No. M-AP(C)-16-5
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Serum Enzymes in Acute Psychotic States

Previous Serial Number: M-AP(C)-16-5

Principal Investigators: William T. Carpenter, Jr., MD.; John S. Strauss, M.D.; Herbert Meltzer, M.D.

Cooperating Units: University of Rochester Medical School, Rochester, New York
University of Chicago Medical School, Chicago, Illinois

Man Years:

Total:	0.2
Professional:	0.1
Others:	0.1

Project Description:

Objectives: In an attempt to determine relationships between biochemical findings and psychotic conditions, Dr. Meltzer has demonstrated that creatine kinase and serum aldolase levels are often elevated in acute psychotic states. The objective of this project is to determine more precisely in which psychotic conditions these enzymes are elevated. By the use of standardized diagnostic interviews to obtain information on a wide variety of symptoms and environmental conditions it will be possible, using a sample of patients to define more clearly the correlations of individual symptoms with enzyme elevations. It will be possible in this way to evaluate how reliably these enzyme methods differentiate schizophrenic patients from other psychotic and borderline conditions.

Methods Employed: A series of patients is being interviewed using standardized mental status, history and social description interviews. Blood is drawn at the time of the mental state interview and analyzed employing methods currently in use by Dr. Meltzer for determining serum aldolase and creatine kinase.

Major Findings: An initial sample of patients has been tested with the techniques described and preliminary findings suggest that degree of "cognitive disorganization" as defined by a group of mental status items, correlates more closely than other parameters with elevated enzyme levels.

Significance to Mental Health Research and Program of the Institute: There is much interest in determining whether schizophrenia and other psychotic conditions can be related to particular biochemical and other physiological measures. Although there is some evidence for such relationships in regard to certain affective psychoses, evidence for similar relationships in schizophrenia is more controversial. Dr. Meltzer has been able to demonstrate relationships between creatine kinase and serum aldolase in psychotic conditions but it is not clear yet to what extent elevations in these enzymes are related to (1) the acuteness of the psychotic conditions, and (2) particular clinical pictures. In the present study, it will be possible to help clarify some of these relationships as well as to evaluate by replication Dr. Meltzer's earlier work.

Proposed Course of the Project: A larger series of patients is being evaluated. On completion of the data collection, symptom patterns will be compared to the results of the enzyme determinations to see whether a meaningful relationship exists.

Serial No. M-AP(C)-16-6
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychiatric History Study: The Nature and Reliability of the Data

Previous Serial Number: M-AP(C)-16-6

Principal Investigators: William T. Carpenter, Jr., M.D., John S. Strauss M.D.

Other Investigators: Ruth E. Boesman, R.N.

Cooperating Units: John Bartko, Ph.D., Biometrics Branch; Nursing Units, 4-E, 4-W, 3-E, 3-W, NIH, Clinical Center; University of Rochester Medical School

Man Years:

Total:	0.1
Professional:	0.07
Others:	0.03

Project Description:

Objectives: (1) To identify different types of data ordinarily obtained in psychiatric history interviewing. (2) To study the reliability for these data as obtained from the patient compared to that obtained from his relative. (3) To study the effect of when in the course of hospitalization data is obtained on the nature of the data elicited. (4) To learn how psychiatric history is obtained in various psychiatric settings. These data will be used to examine the hypothesis that there is a greater variability in psychiatric history information than is usually expected and that the nature of the data obtained relates to the degree of variation.

Methods Employed: Standardized psychiatric history and social description interview schedules developed for the International Pilot Study of Schizophrenia were utilized in interviewing. The first 20 admissions to one of the psychiatric nursing units who also had relatives available as informants were interviewed. The patient and relative were interviewed within one week of admission and again six weeks later. The same interview schedules were used in each interview and the interviewing was rotated between the three investigators listed above in an effort to keep the interviewer as naive as possible to patient information other than that obtained during the interview.

A questionnaire was sent to a large number of facilities that admit psychiatric patients in the United States. This questionnaire requested information about who interviews whom and at what time to obtain psychiatric history information. Data has been collected from 20 patients and their relatives and is being analyzed to determine reliability. Narrative summaries of the interviews and certain key items are being examined by the investigators in order to determine comparability of the less structured aspect of psychiatric history evaluation.

Major Findings: Results show that data obtained by structured questions is most reliable. Several areas of data such as duration of illness, acuteness of onset and precipitating factors have low reliability. These findings challenge the validity of many concepts of psychiatric disorder that are based on results from unstructured collection of these data.

Significance to Mental Health Research and the Program of the Institute: The relationship of past experience to present behavior is fundamental to most psychological theories of behavior and mental function. There is a paucity of data testing the reliability and validity of background information obtained from psychiatric patients and their families. This study will attempt to identify the various means of collecting past history from psychiatric inpatients in various settings and to identify the pitfalls involved in interpreting this information.

Proposed Course of the Project: The findings are being prepared for publication.

Serial No. M-AP(C)-16-7
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Evaluation of Outcome in Schizophrenia

Previous Serial Number: None

Principal Investigators: William T. Carpenter, Jr., M.D.
John S. Strauss, M.D.

Man Years:

Total:	1.1
Professional:	0.8
Other:	0.3

Project Description:

Objectives: (1) To describe the characteristics of outcome in schizophrenia. (2) To study the predictors of outcome in schizophrenia including premorbid history, social background, duration of illness, diagnosis, and symptomatology. (3) To use outcome measures as means for evaluating the validity of different diagnostic systems.

Methods Employed: Two-year follow-up results from the patients seen by the staff of the Psychiatric Assessment Section for the International Pilot Study of Schizophrenia (M-AP(C)-16-1) were rated with scales developed for the study. These outcome results were compared with patient diagnoses as determined by the criteria of the Diagnostic and Statistical Manual of the APA, Kurt Schneider's first rank symptoms, and Langfeldt's criteria for true schizophrenia. Outcome results were also compared with individual symptoms and sixteen items rating areas of premorbid functioning.

Major Findings: Results showed that outcome itself is not a unitary concept, but consists of at least four dimensions: social functioning, symptomatology, need for hospitalization, and ability to work. Each of these variables was independent enough from the others to suggest that outcome represents an open-linked system comprised of several semi-independent variables. This view of outcome was further supported by the fact that each area of outcome was best predicted by the corresponding area of premorbid function: outcome social function by premorbid social function, outcome symptoms by previous duration of symptoms, outcome need for hospitalization by previous hospitalization, and outcome ability to work with previous ability to work. On the other hand, none of the diagnostic systems or individual symptoms were able to predict outcome to a significant degree. Together, these findings suggest that outcome is far more determined by longitudinal dimensions of function than by particular symptoms or symptom patterns.

Significance to Mental Health Research and the Program of the Institute: There has been much doubt whether certain symptom patterns are adequate to define psychiatric "diseases" with predictable outcomes. Such a conception would imply a concept of psychiatric disorder very similar to many medical-surgical illnesses. The current study suggests that this conception of psychiatric disorder is incorrect and that the major determinants of outcome are enduring personality structures rather than a particular "disease" process. This implies that the causes and treatments for prolonged disability might best be aimed at personality variables rather than at a supposed disease process.

Proposed Course of the Project: The initial sample of 140 patients is being augmented by addition of the patients admitted to the 4-East clinical-research ward. More intensive investigation into the functional determinants of outcome will be carried out to understand the outcome process more clearly. Five year follow-up of IPSS cases may be carried out in FY1974.

Publications:

Strauss, J. S. and Carpenter, W. T., Jr.: The Prediction of Outcome in Schizophrenia: I. Characteristics of Outcome. Arch. Gen. Psychiat. 27: 739-746, 1972.

Strauss, J. S. and Carpenter, W. T., Jr.: The Evaluation of Outcome in Schizophrenia. Life History Research in Psychopathology, Vol. 3. Thomas, A., Roff, M., and Ricks, D. F. (eds.), University of Minnesota Press, Minneapolis, 1973.

Presentations: Presented by John S. Strauss, M.D.

1. The Fate of Schizophrenic Patients, presented at the Annual Meeting of the Mental Health Career Development Program. New Orleans, March, 1972.

2. The Evaluation of Outcome in Schizophrenia, presented at the Annual Meeting of the Society for Study of Life History and Psychopathology. New York, April 14-15, 1972.

3. Diagnostic Criteria and Outcome in Schizophrenia, presented at the Annual Meeting of the American Psychiatric Association. Dallas, May 1-5, 1972.

4. Special Session on Predictors and Follow-up in Psychiatric Inpatients, to be presented to the American Psychiatric Association in Honolulu, Hawaii on May 4-12, 1973.

Serial No. M-AP(C)-16-8
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 2, 1972 through June 30, 1973

Project Title: Psychobiology of Cortisol Metabolism

Previous Serial Number: None

Principle Investigator: William T. Carpenter, Jr., M.D.

Other Investigators: Edward B. Fink, M.D.; William E. Bunney, M.D.

Cooperating Units: Section on Psychiatry, Laboratory of Clinical Science,
Section on Cognitive and Perceptual Studies, APB

Man Years:

Total:	.2
Professional:	.15
Other:	.05

Project Description

Objectives: (1) To utilize sophisticated techniques for determining metabolism of cortisol in psychiatric patients. (2) To evaluate manic-depressive patients with a battery of investigations that provide comprehensive information regarding their cortisol metabolism. (3) To investigate the relationship between cortisol metabolism, psychophysiology, and psychopathology. (4) To determine if cortisol variability relates to schizophrenic diagnostic subgroups or outcome.

Methods Employed: Radioisotope dilution methodology was used to determine production rates and metabolic clearance rates of cortisol. Plasma concentration at various hours of the day and 24-hour urinary output of cortisol was determined with routine laboratory procedures.

Standarized clinical assessment techniques are now being employed to give detailed clinical information to relate to steroid variability. Perceptual and cognitive test procedures are being carried out in collaboration with Dr. Buchsbaum.

In a cohort of depressed patients, it was determined that central mechanisms controlling cortisol metabolism functioned abnormally. The mechanisms which determine response to dexamethasone suppression, methopyrapone stimulation, ACTH stimulation and circadian rhythm were intact in depressed patients. However, determining metabolic clearance rates and production rates of cortisol suggests that depressed patients produce more cortisol and metabolize it more rapidly, while maintaining normal plasma concentration. On recovery, these same patients reduce their production rates while maintaining an abnormally high metabolic clearance rate, and, consequently, reduced plasma concentration. These findings suggest two interesting possibilities: (1) That psychological and biological effects of rapid turnover of cortisol may be critical in depressive illness; and (2) the finding of reduced plasma concentration and production rate on recovery while an increased clearance rate is maintained suggests the possibility that relative adrenal insufficiency may be related to the biological vulnerability to depression.

Significance to Mental Health Research and the Program of the Institute: The results of this work have focused attention on the need for sophistication in technical approaches to cortisol research in psychiatry. Static measures alone (e.g., plasma concentration for 24-hour excretion rate) may be misleading about the physiologic activity of cortisol. Findings to date require a reconsideration of cortisol metabolism regarding manic-depressive disorders. The possibility of relative adrenal insufficiency in depressive disorders reopens an area of research which has been dwarfed by the number of findings based on static measures suggesting either normal or increased adrenal function with depressive illness. If there is a biological vulnerability based on adrenal insufficiency, this may be obscured by stimulated cortisol production during the distress of depressive illness. Studying patients during recovered, low stress periods provide an opportunity to identify basic biological dispositions.

Proposed Course of Project: Studies of cortisol metabolism are being incorporated into the 4-East clinical unit's research program. In keeping with the major thrust of the work on 4-East, there will be careful attention to the clinical variables related to altered steroid metabolism and the possibility of using biological variables such as steroid metabolism as validating criteria to test classification systems. The relationship between steroids, psychopathology and psychophysiology will be investigated in collaboration with the Section on Perceptual and Cognitive Studies, APB.

Honors and Awards: None

Publications:

Carpenter, W. T., Jr., Bunney, W. E.: Adrenal Cortical Activity in Depressive Illness, Amer. J. Psychiat. 128: 31-40, 1971.

Carpenter, W. T., Jr., Bunney, W. E.: Dirunal Rhythm of Cortisol in Mania, Arch. Gen. Psychiat. 25: 270-273, 1971.

Carpenter, W. T., Jr., Bunney, W. E.: Behavioral Effects of Cortisol in Man, Seminars in Psychiatry 3: 421-434, 1971.

Carpenter, W. T., Jr., Strauss, J. S., Bunney, W. E.: The Psychobiology of Cortisol Metabolism: Clinical and Theoretical Implications, in Psychiatric Aspects of Medical Drugs, R. I. Shader (Ed.), Raven Press, 1972.

Carpenter, W. T.: Current Status of Serotonin in Affective Disorders, Annals of Internal Medicine 73: 607-629, 1970, as The Proceedings of the Combined Clinical Staffs, National Institutes of Health, Al Sjoerdsma, Moderator.

Presentations:

The Serotonin Depletion Hypothesis in Depressive Disorder, presented by Dr. Carpenter to the Tenth Annual Conference of the Mental Health Career Development Program, Tucson, Arizona, 1971.

Stress, Affective Disorders, and Pituitary-Adrenal Activity, presented to the Ninth Annual Conference of the Mental Health Career Development Program, Tampa, Florida, 1970.

Adrenal Cortical Activity in Depressive Illness presented to the Annual Meeting of the American Psychiatric Association, San Francisco, California, 1970.

Current Status of Serotonin in Affective Disorders, presented to Combined Clinical Staff, National Institutes of Health, Bethesda, Maryland, 1970.

Serial No. M-AP(C)-16-9
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Structured Clinical Interviews: Valid or Not?

Previous Serial Number: None

Principal Investigators: Michael H. Sacks, M.D.
William T. Carpenter, Jr., M.D.

Cooperating Units: John S. Strauss, M.D., University of Rochester Medical School, and John Bartko, Ph.D., Biometry Branch, NIMH

Man Years:

Total:	.2
Professional:	.15
Other:	.05

Project Description:

Objectives: To test the validity of structured mental status interviews used for research and clinical purposes.

Methods Employed: Twenty-eight patients admitted to a NIMH research ward were interviewed with the PSE. Another copy of the same schedule was completed three weeks after admission using data from all available sources. The PSE interview was conducted within this three-week period by a psychiatrist with little or no foreknowledge of the patient, but with extensive experience with the PSE interview. The three-week PSE (3 wk. PSE) was completed by the patient's psychiatrist from information obtained from all sources available to him. This included the patient, his family, members of the staff, and observing the research PSE interview. The two methods of obtaining a mental status were then compared.

Major Findings: (1) The 3 wk. PSE showed 1.6 times as much total pathology; (2) most of this difference could be attributed to a 3.8 fold increase in scores on behavior items, compared to only a 1.2 fold increase in symptom ratings; (3) two subclasses of patients were identified who demonstrated either maximal or minimal differences between the two methods of obtaining a mental status. These subgroups are instructive, and implications for applicability of cross-sectional interviews are being investigated; (4) whether or not significant disparity in assignment of diagnosis will be found between the two data sets on each patient using a computerized diagnostic program (CATEGO) is being investigated. A diagnostic incongruity exists when schizophrenic assignments are made using symptoms (Kurt Schneider's first rank symptoms); (5) cluster analysis results in significantly different cluster assignments of patients depending on whether PSE or 3 wk. PSE data was used.

Significance to Mental Health Research and the Program of the Institute: Structured mental status interviews have become a major instrument in studies of psychiatric patients. They have been used, for example, as basic data collection instruments, in the International Pilot Study of Schizophrenia and in the United States-United Kingdom Study of Psychiatric Disorder. With structured interviews of demonstrated reliability, large amounts of data accumulated by different psychiatrists at different centers can be collected and compared. However, the degree to which these interviews provide data that accurately reflects all information available about the patient's current psychiatric status has not previously been evaluated. The results of the present study suggest that structured interviews significantly underestimate patient psychopathology and this is especially true for observed behavior and in certain types of patients. The difference in patient psychopathology as judged by a structured interview and in an intensive clinical evaluation causes remarkable disparity in category assignment of the same patient using the two data sets. Several systems of classification were employed, but only the clinician-derived diagnosis resulted in satisfactory agreement in patient assignments. Structured interviews lend themselves to use in absolutely reliable, computerized diagnostic systems. The serious problems encountered challenge the validity of this approach.

Serial No. M-AP(C)-16-10
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Recovery Process and Research Data in Acute Psychosis

Previous Serial Number: None

Principal Investigators: Michael Sacks, M.D.
William T. Carpenter, Jr., M.D.

Cooperating Units: John S. Strauss, M.D., University of Rochester Medical School

Man Years:

Total:	.2
Professional:	.18
Other:	.02

Project Description:

Objectives: (1) To define the difference phases of the psychotic process as the patient moves from severe decompensation toward recovery. (2) To describe the kinds of research investigations the patient is able to participate in as he passes through these phases, and the implications of this phasic process for interpreting results of research on psychosis. (3) To examine the relationship of the patients research participation to his therapy.

Methods Employed: Acutely psychotic patients were observed during the course of their illnesses as they participated or failed to participate in different research projects. These observations were supplemented by interviews with the patient, held prior to discharge, regarding his views on the research procedures.

Major Findings: Distinct phases of patient's illness have been identified. The three phases are: (1) The delusional phase during which patients typically were unaware of research expectations. Thought processes are fragmented and delusional. During this time the patient is unavailable for any kind of research or therapeutic activity that involves active cooperation with another person or cognitive engagement in a task. (2) Double-awareness phase during which the patient is still delusional, but has become aware of the ward research and therapy programs. A capacity for insight of his symptoms has begun and the patient begins to modify his behavior despite severe symptoms. Participation and cooperation in complex research tasks, although tedious, are now possible even though delusional interpretations of procedures may suggest a danger to the patient and, in fact, influence the way in which he takes the test. (3)

The recovery phase during which the patient is able to realistically perceive and evaluate the research and his participation in it comes with significant clinical improvement. When the patient fails to cooperate in research procedures, he has clear reasons for refusing -- which reflect his attitude toward the unit, his therapy and his doctor. Themes about the research which were present in delusional form during earlier phases may continue, but are now expressed more rationally in a therapeutic relationship. Research that can be carried out is restricted by the phase through which the patient is passing. In the more disturbed phase, no information is obtainable about either response or other measures requiring active collaboration of the patient; as the patient improves more aspects of his function can be evaluated.

Significance to Mental Health Research and the Program of the Institute: The major focus of experimental research on psychosis in the past has often been on chronic patients. Only recently has greater attention been directed toward the acute patient. This study attempts to define phases of the acute process and to demonstrate their interrelationship to the research findings, the conclusions that can be drawn from them, and the patient's therapy. Identifying the psychological set during collection of data provides a framework for interpreting results which enriches the disease model or simple behavioral rating approach. Awareness of the patient's research experience is vital to the therapist since patients will often utilize their research participation to express thoughts and feelings that otherwise might not be available.

Serial No. M-AP(C)-16-11
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Investigation of the Schizophrenic Process Through Art Productions of Acutely Psychotic Patients

Previous Serial Number: None

Principal Investigator: Harriet Wadeson

Cooperating Unit: 4-East Nursing Unit, Clinical Center, NIH

Man Years:

Total:	.9
Professional:	.8
Other:	.1

Project Description

Objectives: (1) To obtain art productions from acutely psychotic patients at intervals during the course of their hospitalization and follow-up. (2) To utilize the art productions in conjunction with other clinical assessments for the purpose of diagnosis. (3) To aid in the treatment of those patients for whom art expression is particularly beneficial. (4) To compare and study the art productions and the patients' associations to them in order to gain a greater understanding of the schizophrenic process by (a) investigating longitudinal changes by comparing pictures made during acute psychosis, recovery, and follow-up; (b) studying in detail delusions, hallucinations, and other manifestations of schizophrenic thinking as revealed in the art productions; (c) comparing art productions of different patients with one another in order to delineate subgroups of schizophrenic ideation; (d) contrasting the art productions of schizophrenic patients with those of manic and depressed patients.

Methods Employed: All patients on a research ward for the study of acute schizophrenia are evaluated in individual art therapy sessions shortly after admission, just prior to discharge, and at follow-up. Some patients have additional sessions during the course of hospitalization if there is marked change in the patient's condition. Patients are provided with simple media and encouraged to express themselves freely and to free associate to their pictures. At each session they are requested to make the following pictures: (1) whatever they want to draw; (2) a self-portrait; (3) a picture of the illness; (4) pictures of any hallucinations they have had; (5) pictures of any delusions they have had. If there are other pictures that patients particularly want to produce, either during the session or at another time, they are encouraged to do so and to discuss them with the investigator. To date 36 patients have been studied. Each session is recorded on audiotape and a summary is written by the investigator.

Major Findings: Although it is too early to report any solid findings, certain trends have appeared which are being studied further. The parameter of organization in the pictures seems to be particularly revealing of intrapsychic states. The changes in balance between richness and impoverishment in the pictures (the latter is often indicative of depression [H. Wadeson, Characteristics of Art Expression] both within the same patient and among patients provides important data on the process of the illness. Changes in self-concept during the course of psychosis and recovery and differences in self-concept among patients is an important discriminator. Pictures of hallucinations, delusional systems, and other manifestations of schizophrenic functioning are extremely useful in furnishing greater understanding of the patient's experience of his psychosis. Perception of the psychotic experience among different patients at selected times in the course of illness and recovery provides important information.

Significance to Mental Health Research and the Program of the Institute: There is little data of a comparative nature on the art productions of any particular population. Most of the writing in this area is directed toward therapeutic benefits rather than diagnosis and evaluation. Where such reports exist, they are primarily speculative and do not report characteristics of a designated population that has been studied systematically. The purpose of this project is to help counter this lack. In a broader sense, although the outward manifestations of acute schizophrenia are readily studiable, (symptoms, progress, etc.) the inner experience of the affected individual is much less accessible. Hopefully, this investigation will provide data in this area. Finally, in conjunction with other parameters of assessment, it is hoped that the data obtained from the art evaluations will add to the clarity of differential diagnostic dimensions in acute schizophrenia.

Proposed Course of the Project: During the coming year, the sample will be enlarged and the developing trends investigated systematically. Also, new trends will be noted and studied. In addition, the data from this project will be compared with data from art productions of manic and depressed patients, of which the investigator has accumulated an abundance on past projects.

Publications:

Wadeson, Harriet: Characteristics of Art Expression in Depression, J. Nerv. Ment. Dis., 153 (3), Sept. 1971.

Wadeson, Harriet, Fitzgerald, Roy: Marital relationship in manic-depressive illness: conjoint psychiatric art evaluations. J. Nerv. Ment. Dis. 153 (3), Sept. 1971.

Wadeson, Harriet: Conjoint Marital Art Therapy Techniques. Psychiatry, Feb. 1972.

Wadeson, Harriet: Portraits of Suicide, Publication of exhibit, Annual Meeting, American Psychiatric Association, Washington, D. C., May 1971.

Contributions to: Sarason, Irvin: Abnormal Psychology. Appleton-Century-Crofts, New York, 1972.

Wadeson, Harriet: Comment on Sexual Problems Revealed Through Art Therapy, Human Sexuality, in press.

Wadeson, Harriet: Art Therapy Techniques Used in Conjoint Marital Therapy. Amer. J. Art Therapy, in press.

Wadeson, Harriet: Separateness. Art Psychotherapy, in press (1:2, Spring 1973).

Wadeson, Harriet: Hallucinations and Delusions, exhibit and monograph, Amer. Psychiat. Assoc. Annual Meetings, Honolulu, 1973.

Awards: Rush Bronze Medal Award

Special Scientific Recognition

"Here's to Your Health" Radio Program, WGMS, Washington, D. C.

"The Primitive Depressionists," Human Behavior, 1 (2), Mar./Apr. 1972.

"Marriage Counseling - An Easel Way to Domestic Tranquility," Psychology Today, 6 (2), July 1972.

Family Art Therapy Presentation, Strong Memorial Hospital Psychiatric Wing, Rochester, New York, Sept. 1972.

Serial No. M-AP(C)-16-12
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Evaluation of Urinary Bufotenin and Dimethoxytryptamine (DMT) and Serum Methyltransferase in Acute Schizophrenia

Previous Serial Number: None

Principal Investigators: William T. Carpenter, Jr., M.D.
Harold E. Himwich, M.D.
N. Narasimhachari, Ph.D.

Cooperating Units: Galesburg State Research Hospital

Man Years:
Total: .2
Professional: .1
Other: .1

Project Description:

Objectives: (1) In previous investigations, Dr. Himwich has consistently found hallucinogenic substances in the urine of schizophrenics, while absent in normal controls. More recently, his laboratory has found a probable transmethylating enzyme in the serum of schizophrenics, while absent in normals. The objective in this investigation is to test the hypothesis that schizophrenics abnormally transmethylate hallucinogenic compounds.

Methods Employed: Drug-free acute schizophrenic patients subjected to intensive clinical evaluations provide urine and blood samples for biochemical determinations. Nonschizophrenic patients and normal volunteers provide control urine and blood specimens. The schizophrenic patients are studied during sick, recovered and follow-up periods. Biological specimens are shipped frozen to Galesburg, Illinois for assay.

Major Findings: Preliminary results from the first several subjects are not compatible with the hypothesis.

Significance to Mental Health Research and the Program of the Institute: The development of a clinical methodology and the generation of an adequate patient group to test major biochemical hypotheses of schizophrenia has wide-ranging implications. Positive findings of theoretical significance in such a group of patients would greatly increase interest in that particular hypothesis. Clear refutation of previous work, when done with rigorous clinical methodology, can help reshape hypotheses and require reconsideration of a broader range of variables in attempting to account for schizophrenic illness.

Proposed Course in Project: This project will continue until sufficient sample size permits reaching conclusions. Future development of the work beyond that will depend on the findings.

Serial No. M-AP(C)-16-13
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Computerization of DSMII

Previous Serial Number: None

Principle Investigators: William T. Carpenter, Jr., M.D.

Other Investigators: John J. Bartko, Ph.D.
Christine Diana Nicholl, Ph.D.

Man Years:

Total:	.6
Professional:	.4
Other:	.2

Project Description

Objectives: (1) To empirically evaluate the success of DSMII as a diagnostic manual; (2) To provide a simple computerized program using Present State Examination data to produce "American" diagnoses with the absolute reliability of a computerized diagnostic system; (3) To employ this system in cross-cultural comparisons.

Methods Employed: The Present State Examination (PSE) contains approximately 360 items indicating the presence or absence of signs and symptoms. Using DSMII as an instructional manual, each PSE item was judged as to whether or not it was discriminating for any diagnostic group. Once these judgments had been made, a computerized diagnostic tree was constructed.

Major Findings: Preliminary work has indicated that such a system is feasible to construct, and diagnostic assignments give a reasonable distribution of our patient groups, according to clinical diagnosis and expected distribution of patients. Work on refining this diagnostic system is continuing prior to any effort to apply it to the problems of diagnostic classification.

Significance to Mental Health Research and the Program of the Institute: With data collected from nine different countries using the PSE Interview Schedules, we have an opportunity to construct computerized diagnostic systems based on American and European diagnostic approaches which empirically evaluate the potency of each system in predicting outcome or postdicting premordid factors in the functional psychoses. Furthermore, once functioning, this system will provide us with a way to reliably assign our patients to diagnostic categories where the rules for classification are clearly spelled out.

Proposed Course of the Project: Once this system is functioning to our satisfaction, we will use it to diagnose patients from the various countries of the IPSS and determine to what extent they relate to clinical diagnosis given by the interviewing psychiatrist and computerized diagnosis given with systems based on European diagnostic approaches. Potency of predicting outcome will be evaluated comparatively for each system. If possible, we will collaborate with USSR diagnosticians in computerizing their unique approach to classification and contrast DSM II with their system.

Serial No. M-AP(C)-16-14
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical Delineation of the Borderline Syndrome

Previous Serial Number: None

Principal Investigators: William T. Carpenter, Jr., M.D.
John Gunderson, M.D.
Winfield Scott, Ph.D.
John S. Strauss, M.D.

Man Years:
Total: .2
Professional: .1
Other: .1

Project Description:

Objectives: (1) To describe the borderline syndrome in operational terms; (2) to determine similarities and differences in premorbid factors, prognostic indicators, symptom profiles, and outcome with schizophrenic patients.

Methods Employed: Patients from the Washington Field Research Center of the International Pilot Study of Schizophrenia (see M-AP(C)-16-1) were used for this investigation. Those who met our criteria for the borderline syndrome were identified and doubtful cases eliminated. This resulted in a group of 27 patients diagnosed as borderline who were then matched with a group of schizophrenic patients where diagnostic certainty was reasonably assured. Smaller groups of manic and depressive patients were used for comparative purposes. Psychiatric history data, prognostic items, signs and symptoms, and outcome measures had been systematically determined in these patients.

Major Findings: It was possible to identify a group of patients hospitalized with borderline psychosis. This group bears important similarities to schizophrenia in outcome and premorbid factors, while differing in symptom picture. We are determining whether or not Rorschach material can distinguish between these groups.

Significance to Mental Health Research and the Program of the Institute: Borderline syndrome has not been adequately defined in operational terms so that it is largely used as a wastebasket diagnosis. Systematic investigation of the borderline syndrome provides an opportunity to clarify similarities and differences between schizophrenic patients and borderline patients. The implications for generation of homogeneous groups for various research purposes and possible treatment implications based on accuracy of diagnosis are significant contributions which may accrue in this work.

Purposed Course in Project: Examination of Rorschach material in the schizophrenic and borderline patients is being undertaken. We will attempt to operationalize the distinction between borderline and schizophrenia so that it can have general diagnostic applicability.

Serial No. M-AP(C)-17-1
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Study of Problems in Growth and Adaptation in the Personality Development of the Adolescent

Previous Serial Number: Same

Principal Investigator: Roger L. Shapiro, M.D.

Other Investigators: Carmen Amoros-Cabrera, M.S.W.; David Berkowitz, M.D.; Burton Schonfeld, M.D.; Edward Shapiro, M.A., M.D.; Robert Winer, M.D.; John Zinner, M.D.

Cooperating Units: Unit 3-West

Man Years:
Total: 7.65
Professional: 4.05
Other: 3.60

Project Description:

Objectives. The over-all objectives of this study continue to be investigation of individual adolescent personality development in the context of his family and of his social group. This investigation is done through study of a number of clinical situations in a residential treatment unit for adolescents where intensive work with families is also done. We are evolving a typology of families derived from observation of their interaction in conjoint family therapy. In particular, we are characterizing families of adolescents with borderline symptomatology, adolescents who do antisocial acting out and adolescents who are severely neurotic.

We are in addition studying group life of the residential treatment unit. Peer relations and authority relations in the inpatient group are studied in the weekly unit study group meeting; the relations of all the families to each other and to the staff are studied in the weekly multiple family meeting. These are the situations in which we observe roles taken and given to individual adolescents as well as to families in a social context. These observations are central to our investigation of individual personality formation in the adolescent and its relatedness to group roles and family characteristics.

Methods. Study of families of borderline adolescents, of antisocial acting-out adolescents, and of neurotic adolescents is currently being done through observation of conjoint family therapy, of marital therapy of the parents and of intensive individual therapy of the adolescent. Excerpts of family interaction containing defensive delineations of these adolescents by the parents provide evidence about the psychodynamic characteristics of the family and the unconscious assumptions of the family as a group. Data from the individual psychotherapy of the adolescent is the basis of formulations about the state of the ego in the adolescent, his capacity for object relations and level of defensive organization.

The unit study group is a weekly meeting of all inpatients with the project director, unit administrator, and chief nurse. The relations of the adolescents to authority figures and to peers are studied in this meeting. The roles adolescents take and are given in the ongoing life of the residential treatment unit are articulated. The relation of these roles to roles they take and are given in their families are studied.

The multiple family meeting is a weekly 75-minute meeting of all of the families of the inpatients, the project director, the administrator, a psychiatrist in a specifically designated research role, and the evening nursing staff. The task of the meeting is to study the relations of the families to the hospital staff and to each other. This is a large group of 40 to 50 people with a number of clear subgroups. The behavior of individual families as subgroups is one focus of study; the staff as a subgroup is another. In addition, the adolescents are seen to be a subgroup often divided between inpatients and their outpatient siblings; and the parents are another subgroup often divided into fathers and mothers.

In this meeting behaviors are studied by which families either maintain or diffuse family boundaries in subgroup formation within a larger social milieu and relate in a variety of ways to the rest of the social milieu. Attitudes of parents as well as adolescents toward the authority of the staff are studied. A focus is made on parental acting out in relation to staff authority in order to explore dynamic parallels to adolescents acting out in relation to parents. The conceptualization and technical method for conducting this investigation and working in this meeting are an application of methods learned by conducting large groups in Tavistock-A. K. Rice Institute Group Relations Conferences.

Major Findings. Comparison of families of borderline adolescents, antisocial acting-out adolescents, and neurotic adolescents reveals clear differences in the psychodynamic mechanisms determining defensive delineations of the adolescent by the parents. Parents of borderline adolescents manifest primitive splitting and projection of badness as the dominant mechanisms underlying defensive delineations of the index adolescent. In our series this is true for at least one of the parents; the parent is the parent most involved with the adolescent; the other parent may not manifest the same dynamic in delineation but is regularly acquiescent to the splitting and projecting parent and does not provide a corrective experience for the index adolescent. Unconscious assumptions dominated by splitting and projection are inferred in these families. Frequently these assumptions are fantasies intended to control expression of sexual or aggressive impulses which are seen as bad and destructive and which must be denied within the family. Frequently assumptions of compliance and sameness are required of family members with the family boundary then containing what is good and the bad being projected outside the family. If the adolescent differentiates himself from other family members in his expression of impulses or ideas, he becomes bad and suffers feelings of extreme alienation from other family members.

Parents of antisocial, acting-out adolescents are more differentiated than the parents of borderline adolescents. Projective mechanisms are the basis of defensive delineations, but the projections are less massive and are likely to be differentiated aspects of impulse life like particular forms of sexual behavior (e.g., promiscuity, homosexuality) with more differentiated superego responses being retained within the parents. In addition to being more differentiated, the projections are more easily taken back by the parents during the course of family therapy. Unconscious assumptions are relatively differentiated fantasies which contain anxieties and concerns of the parents rising from their own internal conflicts, with one element in the conflict being projected into the developing adolescent. These unconscious fantasies differ from family to family but contain in them fantasies originating in one or both parents (e.g., fantasies of promiscuity, homosexual fantasies, etc.) which are projected by the parents into the adolescent. Covert gratification of these impulses in the parent or parents then occurs through their identification with the adolescent.

Parents of neurotic adolescents employ predominantly repressive defenses rather than projective defenses. In their defensive delineations of the adolescent they remain oblivious to major areas of behavior in the adolescent which would otherwise be conflictful for the parents. Their behavior toward the adolescent is frequently dominated by repression of conflict at the Oedipal level in themselves, leading to repressive defenses in delineation of the adolescent in areas of sexuality, competition, and competence. Unconscious assumptions of the family as a group may be inferred which have differentiated and elaborated Oedipal themes.

In individual therapy and in the Unit Study Group (as well as in family therapy itself) the adolescent struggles to find a new role in relation to therapist and to peers and authority figures on the unit as he grasps the implications of the role he has been put into and has taken in his family. He resumes an adolescent development which had been interfered with because of his idiosyncratic family role.

The multiple family meeting facilitates this process. In this meeting parents and adolescents can achieve an objectivity through observing the behavior of other families which is difficult for them to achieve in the situation of conflict within their own family therapy. Adolescents can see problems over responsibility for personal behavior in their parents as well as themselves. The meeting is a social situation which the need for defense out of social anxiety and shame is gradually mitigated, facilitating an increase in the assumption of individual responsibility in family members.

Significance to Mental Health Research. As a typology of families of borderline, antisocial acting-out, and neurotic adolescents is articulated in this work, it is clear that it has a general importance beyond knowledge about the family behavior underlying disturbance in adolescence. This work suggests that it is important to understand emotional disturbance in the context of family and group dynamics rather than to conceptualize disturbance as the fate of an isolated, "sick" individual. This understanding of disturbance in an individual as a manifestation of disturbance in a family counters the tendency in families and in the community to scapegoat individuals by isolating them as sick and attempting to locate all disturbance in them. This work constitutes evidence against the current unfortunate and reactionary tendency in psychiatry to isolate individuals who are emotionally disturbed and to consider them sick in a way which tends to exclude consideration of the families in which they develop and the society in which they live. Consequently this study has important implications both for the prevention of emotional disturbance and for family and community programs of treatment.

Proposed Course of Project. In the next year it is anticipated that current work with families of borderline, acting-out, and neurotic adolescents will be terminated because of changes in branch leadership. During the next year there will be a phasing out of the clinical program, and several new publications on the findings outlined above will be completed .

Honors and Awards:

Roger L. Shapiro, M.D., Principal Investigator

Director, Conference on Group Relations sponsored by The A. K. Rice Institute of The Washington School of Psychiatry, Amherst College, August 25-30, 1972.

Director, Conference on Group Relations sponsored by The A. K. Rice Institute of The Washington School of Psychiatry, Mt. Holyoke College, June 1-10, 1973.

Senior Staff, Conference on Group Relations sponsored by The A. K. Rice Institute and the Menninger Foundation at the Menninger Foundation, Topeka, Kansas, January 6-10, 1973.

Senior Staff, Conference on Authority and Leadership sponsored by the Centre for Applied Social Research of the Tavistock Institute of Human Relations, London, England, at the University of Leicester, April 6-19, 1973.

Publications:

Zinner, J. and Shapiro, R. L. Projective Identification as a Mode of Perception and Behavior in Families of Adolescents. Int. J. of Psychoanalysis, 53(4), 1972.

Zinner, J. and Shapiro, E.R. Splitting Phenomena in Families of Borderline Adolescents. To be published in Seminars in Psychiatry.

Presentations:

Shapiro, R. L. Discussion of paper entitled Group Psychology and the Structural Theory: A Revised Psychoanalytic Model of Group Psychology, by Stephen Saravay, M.D. To be presented at the 60th Annual Meeting of the American Psychoanalytic Association, Honolulu, Hawaii, May 4, 1973.

Shapiro, R. L. Family Treatment of Adolescent Disturbance. Presented at a meeting of The Washington Society for Adolescent Psychiatry, Panel on Treatment for the Adolescent, November 29, 1972.

Shapiro, R. L. Participant on Panel Discussion of The Psychoanalytic Theory of Schizophrenia, at a meeting of The Washington Psychoanalytic Society, May 18, 1973.

Zinner, J. and Shapiro, R. L. The Family as a Single Psychic Entity: Implications for Acting Out in Adolescents. To be presented at the 60th Annual Meeting of the American Psychoanalytic Association, Honolulu, Hawaii, May 4, 1973.

Zinner, J. and Shapiro, R. L. Progressive and Regressive Aspects of Peer Group Interaction among Adolescents. To be read at a Panel on Wandering Youth's Efforts at Self-Help at the American Society for Adolescent Psychiatry, Honolulu, Hawaii, May 6, 1973.

Zinner, J. Changing Life Styles. Presentation at the Annual Meeting of the Community Psychiatric Clinic, Bethesda, Maryland, May 10, 1972.

Serial No. M-AP(C)-17-3
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Psychogalvanic Response in Family Therapy

Previous Serial Number: Same

Principal Investigator: John Zinner, M.D.

Other Investigators: David Reiss, M.D. and Roger Shapiro, M.D.

Cooperating Units: Experimental Group and Family Studies Section, APB;
Unit 3-West

Man Years

Total: 1.25
Professional: .50
Other: .75

Project Description:

Objectives. The family psychophysiology project was initiated to provide quantifiable information about subjective experience during family interaction that would supplement self reports of family members and inferences made from observable behavior.

Studies of interaction in families of emotionally disturbed adolescents carried out by this section in the past and concurrently have consistently focused attention on the subjective experience by family members of one another as important determinants of family group behavior. In particular, we have found clinically that family members often behave in such a way as to reduce anxiety in the family group as a whole or within particular members. In the case of emotionally disturbed adolescents the psychopathologic outcome may derive at least in part from the patient's attempts to diminish parental anxiety over parental intrapsychic conflict.

Because family members may not be aware of anxiety during family sessions and because anxious members may choose to remain silent and not report their feelings or demonstrate it overtly in their behavior, we undertook to monitor continuously autonomic indicators of anxiety during family therapy sessions. The study therefore adds a psychophysiologic dimension to our ongoing investigations of the interplay between subjective experience and behavior in family interaction. Since the unit of study is conjoint family therapy, an opportunity is provided for exploration of the process of family therapy within individual sessions and on a long term basis, as well as the physiologic reactions of family members to the interventions of the psychotherapists

Method. The sample consists of families of adolescent patients admitted to our treatment program as well as families of "normal" adolescents interviewed in the "Sidwell Project."

The psychophysiologic variable selected for initial study was the galvanic skin response (GSR) which is known to be a rather good indicator of anxiety occurring in an interpersonal context. In addition, the GSR has the value of being discrete, unidirectional, easily visualizable and proportional in amplitude to the experienced intensity of the emotional stimulus.

During conjoint family interviews, the GSR of family members and therapists are monitored continuously on a Grass Polygraph by way of electrodes attached to two fingers of each subject's non-dominant hand. A timing device and polygraphic recording of speech permits the accurate synchronization for retrieval of physiologic responses and concurrent verbal behavior. In addition, sessions may be video-taped using a split-screen technique which records and displays simultaneously both the family in interaction and the polygraph record. Playback of these videotapes permits an analysis of the sequential unfolding of family behavior and subjective experience of family members.

Progress and Major Findings. A small sample of families of "normal" and "hospitalized" adolescents has been monitored physiologically during family sessions. Each family has been tested on at least two occasions to evaluate the stability of the physiologic variables. There is a close relationship, between occurrence of GSRs and the operation of psychological defense mechanisms. GSRs can be peripheral indicators of central anxiety "signals." These are subjective phenomena which stimulate the operation of psychological defenses preventing the eruption of a greater intensity of anxiety. Psychological defenses which limit anxiety limit the further occurrence of GSRs. In the family therapy situation, however, there are stimuli beyond the control of the family, such as therapists' interpretations, which challenge defense mechanisms and generate anxiety, with a corresponding increase in GSR amplitude and frequency. Individuals in whom anxiety is, in general, poorly bound demonstrate marked degrees of GSR activity on polygraph records. At the other end of the spectrum are those individuals with hypertrophied defense operations, marked ego restriction and almost quiescent electrodermal activity. Thus the physiologic recordings, conform to psychoanalytic concepts of anxiety which postulate that primitive arousal mechanisms are incorporated and modulated by the ego so that they may performa signal function for the operation of psychological defenses.

The investigators had planned this year to use electrodermal variables to test hypotheses derived from our theory of family interaction which relates family group behavior to defensive needs of individual members. This would have involved approximately one year of data collection on a new carefully selected sample of families from 3-West. Because of the profound uncertainty over the fate of the Adult Psychiatry Branch, it was decided not to embark on extensive data collection, the analysis of which was quite likely to be interrupted. In addition, plans for collaborative studies using physiological variables in an investigation of alchoholism (with Dr. Peter Steinglass) and of body movement (with Dr. C. Christian Beels) had to be postponed.

Attention was focused on the pilot sample of families which had previously been monitored, with physiological and speech variables scored. There was great variability in level of arousal of family members depending on who was speaking to whom within family sessions.

Our prediction that certain "who speaks to whom" interactions would have consistently high associated arousal levels across sessions within families was not, in general, confirmed. This finding can be accounted for by our observation that the most effective suppressors of PGR activity are the psychological defenses; the degree to which family members employed these defenses varied considerably across sessions and these variations would wash any expected consistency in the "potential" that one individual has for arousing another.

Serial No. M-AP(C)-17-4
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Follow-Up Study of 37 Families Treated in the Study of Adolescent Identity

Previous Serial Number: Same

Principal Investigators: Carmen Amoros-Cabrera, M.S.W. and Roger Shapiro, M.D.

Other Investigators: David Berkowitz, M.D.

Cooperating Units: Unit 3-West

Man Years

Professional: .10
Other: .05
Total: .15

Project Description:

Objectives. To obtain information about the current adjustment of the adolescent and his family as related to (1) the problems that motivated their admission to the project, and (2) their individual perceptions of the effects of therapy to any changes that may have taken place.

Methods Employed. The family group, the adolescent, and the parents are seen in separate interviews with the goal of providing information which will allow for the isolation and identification of various factors comprising the therapeutic experience, such as the impact of the adolescent's hospitalization, ward milieu, family therapy, couples therapy, and individual therapy. The outcome of each aspect of the therapeutic program is emphasized, and outcomes will be evaluated for a number of categories still being defined.

Progress. Sixteen families have been seen in the follow-up study this past year, and a paper was written on the preliminary findings focusing on couple's therapy with the parents and their status at follow-up interview. While adolescence and adolescent turmoil are frequently seen as an opportunity for the growth and development of the child, little attention has been given the possibilities of change for the parent during his child's adolescence. The findings of our study indicate that parents, precipitated into couple's psychotherapy by the adolescent turmoil of their children, have considerable capacity for psychic change at that time.

On the basis of follow-up interviews and evaluation of data from long-term therapy with 22 parental pairs, three stages of regression and progression through which these couples pass during the therapeutic process were defined: (1) loosening of ties to the original family, (2) renewed adolescence and early marriage, and (3) return to parenting.

Of the 27 follow-up cases, there was evidence of drug use in 11 of the index patients (the adolescent). Using a scale of -5 to +5 to evaluate adjustment, each adolescent was rated for factors such as capacity for independent living, socialization, heterosexual adjustment, ability to handle finances, productive application of time and energy, and sustained level of health. The results were:

1 case	+5
5 case	+4
1 case	+3
1 case	+1
1 case	0
1 case	-3
1 case	-4

of these cases, six continued in therapy after discharge as we recommended. Five of them had married and seemed to be functioning adequately in relation to their family responsibility; 4 of the 11 had completed college and were successfully employed. Two adolescents showed no indication of progress. One continued to be extremely delinquent, a child of very unstable parents, and his involvement with drugs has become increasingly serious. The other had presented clear indications of psychotic content since admission, had shown marked recovery periodically, but had failed to sustain an adequate level of functioning and was hospitalized.

Significance for Mental Health Research. We are particularly interested in learning about the success of the therapeutic plan in clarifying the index patients' identity problems and in understanding and modifying the parents' delineations of the adolescent. This study will be useful in isolating factors contributing to the precipitation of serious adjustment problems during adolescence and in evaluating the outcome of a treatment program derived from research findings.

Proposed Course of Project. On the basis of the information obtained so far, a paper was developed. The title is the"Effect of Adolescent Turmoil on Parental Growth and Development: A long Time Follow-up of Couple's Therapy." This paper was presented in September 1972 to the Adult Psychiatry Branch, NIMH and is at present being edited, hopefully for publication.

Additional families will be added to the sample. A review of the information obtained indicated the advisability of modifying some aspects of the research strategy in order to obtain more useful data.

Serial No. M-AP(C)-17-5
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cognition and Identity Development in Early and Late Adolescence: Longitudinal Studies

Principal Investigator: Stuart T. Hauser, M.D.

Other Investigators: Roger L. Shapiro, M.D.

Cooperating Units: Ward 3-West, Normal Volunteer Office

Man Years:

Total: .2
Professional: .1
Other: .1

Project Description:

Objectives. An earlier longitudinal study of identity formation in lower socio-economic class boys (Hauser, S. T., Black and White Identity Formation, N. Y., Wiley, 1971) suggested several areas for further investigation. A clear difference in identity formation patterns was found between the black and white adolescent subjects. Two questions of interest emerging from this earlier investigation and orienting the current studies are:

1. What variants of identity formation are found in other groups of adolescents, such as girls, other social classes, and psychiatric patients?

2. What are other aspects of psychological functioning which are associated with varying identity formation patterns? In particular, cognitive processes appear to be related to modes of self-image perception. How then are these cognitive areas linked with identity development patterns, more complex patternings of self-images? Are certain cognitive or perceptual processes more powerfully linked to identity development?

Methods Employed. To approach the preceding questions, several populations of subjects are being sampled:

1. Adolescent male and female patients of middle and upper socio-economic class status (24 subjects).

2. Adolescent male and female high school and early college age students, also of middle and uppersocio-economic class status (35 subjects).

Each of the subjects from these samples is studied longitudinally for two years, being seen every six months. The sequences of meetings and instruments used, with rationale for their use, is the following:

1. Groups of seven to eight subjects from each of the samples meet with the senior investigator for a series of four meetings, focused around "the study of roles and perceptions of the self and others in the group."

2. From the tapes of these meetings, a separate Q-sort deck is generated for each group. The deck consists of 3x5 cards, on each being an "I" statement made by a group member which reflected a particular personal emotional, ideological, or general descriptive position about himself. There is an equal number of statements from each group member, including the leader.

3. Each subject is individually interviewed following the conclusion of the four group meetings, the thrust of the individual sessions being around self-conceptions, current and past. Inquiry is also along the lines of developmental and family history.

4. The following week the subject takes the Q-sort task. In this method he is asked to sort the deck from his group (described in #2 above) for a series of seven different self-images. These include self-images from temporal idealized, and peer realms. There are a total of seven self-images, always requested in the same order by the experimenter.

5. In the following testing one to three weeks after the Q-sort, the subject is tested with a series of cognitive measures. The techniques, their rationale and their order of being taken are:

a. The Pettigrew Category Width Scale. This is to measure the breadth or narrowness of a subject's categories.

b. The Concealed Figures Test. For determination of degree of field articulation, of "differentiation."

c. The Object Sorting Test. To determine the size and number of categories generated by a subject; and, in addition, to analyze his reasons underlying his categories (conceptualizations).

d. The Twenty Questions Task. To measure the type and degree of efficiency of a subject's problem-solving strategy.

e. Draw-A-Person Task. The drawing of persons obtained will be analyzed using the scales devised by Witkin and Associates for measurement of"sophistication of body image," a variable highly associated with differentiation.

Steps three through five are repeated every six months for a two-year period. Thus each subject will be interviewed and tested a total of four times.

The self-image measurements which are being obtained from these instruments include correlational indices, general measures of relatedness and discrimination (all obtained from the Q-sorts); indices of the sources of self-image components (also from the Q-sorts). Specific cognitive variables from each of the cognitive instruments are simultaneously being determined.

Techniques for analyzing the complex data is along several lines in order to investigate inter-relationships of the variables at single points in time, and over time; and to investigate consistencies within individual subjects and between groups of subjects. Two basic statistical tools will be analyses of variance for repeated measures, and factor analyses. Both these approaches and their implementation have been worked out with Dr. John Bartko, statistical consultant, and the computer center.

A new technique of analysis, developed with the collaboration of the late Dr. William Caudill, is a content coding system which is being applied to each interview. Five minute segments are used as the units of analysis. The content categories are defined along self-image, cognitive, and affect (e.g. self-esteem) lines. Approximately one half of the interviews have now been analyzed. High reliabilities among three coders have been found.

The data (frequency of coded categories in individual interviews) will be analyzed by age, sex, and psychiatric status using repeated measures of analysis of variance techniques. This quantitative data, and more qualitative analyses of the multiple interviews will be compared to the extensive analyses of the Q-sort self-image results thus serving as a means of further clarifying empirical meanings of the Q-sort.

Major Findings. At this point four waves of interviews and testings have been completed on all the controls and most of the patients. The data obtained from the first round has been analyzed for measures noted earlier. Correlational analyses of the Q-sort data are completed and have been written up in "The Differentiation of Adolescent Self-Images," presented at the annual American Psychiatric Association meetings and to be published in Archives of General Psychiatry. The major finding is in the differential sensitivity of various self-images (idealized, temporal, peer) to variables of sex and patienthood. Implications of these differences are being studied, and further elaborated. One further elaboration concerning the structural complexity of the self-images found normal boys to be a very specific sub-group having highly polarized images. This result is being published in J. of Youth and Adolescence.

A second aspect of the sample is in the black-white comparative studies. Data from the original New Haven sample was re-analyzed using new tools: information statistics (for analysis of structural complexity of self-image and over-all relatedness) and drawing analyses for study of differentiation. The structural complexity analysis of the older longitudinal data is consistent with lessened structural complexity (more polarized) self-image for several of the Blacks' images. These results were published this year in Archives of General Psychiatry.

Significance to Mental Health Research. These studies are directed toward an increasingly intensive analysis of self images from several perspectives: Their "inner" characteristics of meaning and structure, their relation to one another, their modes of change over time, their relation to other modes of personality functioning which appear highly relevant (such as cognitive processes). One important application of such quantitative studies is as a means of following the course of an adolescent during psychotherapy comparing other measures with independent observations such as those of nurses or the patient's therapist. A second application is to the comparison of adolescent development with and without psychotherapy, and under different modes of psychotherapy (half the patient population is receiving only group therapy, the other half is in group and individual therapy).

The operationalizing of major concepts such as "identity formation" and "psychosocial moratorium" is still a third important consequence of this kind of research. Through such operationalizing the concepts are given clearer empirical definition and rendered more susceptible to rigorour quantitative research. Moreover, the way is opened to study of these much discussed phenomena (identity development) in many varied groups ranging from ones within our own culture--ethnically, racially, psychiatrically, socioeconomically differentiated-- to cross-cultural comparisons.

Course of the Research. The plan was to complete data collection for a two-year period on all of the subjects. This has involved four waves of interviews and testings on each subject; because of the different dates of admission, six patients remain to have their data collection completed. The data is being analyzed using several analysis of variance models: two and three-way ANOVA's for cross-sectional, and repeated measures model for longitudinal. A second facet of the research is content analysis of the interview materials along the lines of different self-images and their changes, as described above.

A preliminary outline for the eventual integration of the findings in monograph form,"Lines of Adolescent Development," has been written up. Specific discussions of new methodological and substantive findings during the progress of the studies are complete. These are summarized in papers listed below. The first of these papers, "The Differentiation of Adolescent Self-Images," is to be presented at the annual APA meetings.

Publications:

1. Hauser, S.T. Black and White Identity Development: Aspects and Perspectives, Journal of Youth and Adolescence I: Summer 1972.

2. Hauser, S.T. The Development of Adolescent Self-Images, *Archives of General Psychiatry*, 27: 537-541.

3. Hauser, S.T., and Shapiro, R.L., The Differentiation of Adolescent Self-Images, *Archives of General Psychiatry*, 1973, *in press*.

4. Hauser, S.T., and Shapiro, R.L., "Dinemsions of Adolescent Self-Images, *Journal of Youth and Adolescence*, 1973, *in press*.

Serial No. M-AP(C)-18-2
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Coordinating "Micro-Codes" in Family Consensual Experience: A Study of the Responses to Speech Hesitancy and Fluency in Family Interaction.

Previous Serial Number: M-AP(C)-18-2

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.1
Professional:	.0
Other:	.1

Project Description:

Objective: The card-sorting experiment and previous studies by Reiss as well as by Strodtbeck, Mishler, and Waxler, et al., support the notion that families can be distinguished by the shared experience, of their members, of their environment. Furthermore, it appears as if this shared experience serves as a major regulator of moment-by-moment family interaction in a variety of settings. In order to maintain such consensual experience and to utilize it to deal with environment events as they occur on a moment-by-moment basis, family members must be exquisitely sensitive to each other's ideas and feelings at all times. The project was part of a card-sorting experiment in which family members could communicate only by voice; thus each member had to utilize the other's vocalizations to remain continuously integrated in this delicately balanced family process. Recent experimental evidence suggests that family members may utilize changes in each other's patterns of speech hesitancy and fluency for this purpose. We postulated that all families will show significant moment-by-moment response to changes in the fluency patterns of each member. However, some families--which we have termed "environment-sensitive"--are predicted to respond to hesitant speech as a signal pointing to a difficulty or incongruity perceived by the speaker in the laboratory puzzle itself. Other families--we call them "consensus-sensitive"--will interpret hesitancy as a sign of the speaker's perception of or response to the family itself. These family types are defined by a consensual experience model and identified by their non-verbal performance on a card-sorting procedure. Thus, this study is a further test of the consensual experience model which predicts that family members will respond to minimal changes in each other's speech in ways determined by their over-all shared experience of the environment. These minimal speech changes could then be viewed as a personal, family "micro-code" serving to regulate its interaction in moment-by-moment harmony with its shared experiences and views.

Method: The verbal interaction in the second family problem of the card-sorting task was recorded on a specially prepared 4-channel tape recording system and transcribed by polygraph to give a visual, objective record of patterns of vocalization. A timing signal permitted coordination of the polygraph record with a typescript of the content of the family's discussion. Changes in fluency were measured by methods of Boomer and Dittman, and Goldman-Eisler, using the polygraph record. The family's response to fluency changes in any member was measured using interaction codes developed by Reiss and Mishler and Waxler, using the typescript, and also measures of the card sort and trial times.

Progress: Data collection is continuing.

Serial No. M-AP(C)-18-3
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: The Effects of Progressive Isolation of an Individual From His Family on His Perceptual Functioning: Use of a Teletype-LINC Apparatus to Study the Reciprocal Relationship of Family Interaction and Individual Thinking.

Previous Serial Number: M-AP(C)-18-3

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.1
Professional:	.0
Other:	.1

Project Description:

Objective: Previous experiments in this Section have demonstrated that family interaction does have a specific, predictable short-term effect on the thinking and perception of its members. A critical question is: of all the flux and nuance of family interaction, which aspects are the most critical for regulating the thought and perception of its members. In the card-sorting and micro-code experiments, Dr. Reiss explored the role of various details of the vocalized portion of family interaction--excluding all forms of non-vocal performance. In the current experiment, the question is: to what extent do families differ in the amount of objective information--concerning the problem solution--they can obtain and distribute among its members; and, further, how does this obtained and distributed information regulate perceptual processes in their members. In other words, in what way does an individual's perceptual process--occurring in the midst of a family problem-solving task--depend on the quality and quantity of information provided him about the problem by his family, disregarding all other aspects of the family interaction of which he is a part. It is predicted that families will differ in the amount of relevant information they can discover about the problem, their effectiveness in distributing it to all members of the family and the use each member makes of information provided to him by others in the family. Specifically, we expect families we have designated as environment-sensitive will extract a great deal of problem-relevant information from their environment, will effectively distribute it amongst members in the family and each member will utilize the information insofar as it assists him a logical, consistent and comprehensive problem solution. Individuals in families designated as consensus-sensitive will work to obtain, distribute, and utilize problem-relevant information only insofar as it permits a family solution at a desired level of within-member agreement. In the present experiment the procedure progressively isolates each member from his family. In the first condition all information obtained by all members about the problem is transmitted immediately to each individual along with the identity of the family member who obtained it--the "public mode"; in the second condition the information is transmitted after a variable delay without the identity of the member who obtained it--the "anonymous mode"; in the third condition no information is transmitted between members--the only information available to each member is what he obtains himself and that provided by an impersonal, extra-family source--the "standard mode." It is predicted that members in environment-sensitive families will maximally utilize information from any source whereas members in consensus-sensitives will utilize information from their past when it comes from an impersonal source. In order to provide immediate and standardized information transmission between the family members and between the family and the impersonal environment a teletype-LINC apparatus was constructed and programmed. This permitted the conduct of experiments entirely under the control of computer. It further permitted every item of family interaction, i.e. transmission of information by use of teletypes, to be precisely recorded in time. Thus, at the conclusion of the experiment it is possible to know not only what interaction has occurred but precisely when. This will permit an exact correlation of the various events comprising the flux of family interaction in this experimental setting. The teletype-LINC apparatus represents a major innovation in experimental social psychology and this project, in addition to its exploration of a particular hypothesis about family interaction, serves as an initial study of this innovation.

Method: Four family members, the parents and two children, are seated in booths so they cannot see each other. Each has a teletype and is instructed in its use. They are told that they must discover a class of symbol sequences that, when typed, will result in plus (+) being typed in return--automatically. Their understanding is that the plusses are typed in a systematic way and their job is to discover the system or pattern. Each member is given an example (e.g. CSTTTTTTTS which represents the class of "plus sequences" beginning with CS followed by *n* number of Ts and concluding with an S). Then each member tries out his own sequences in turn. In the "public mode" each sequence is automatically typed, immediately, on the other members' teletypes, with a symbol indicating whose sequences it was and whether it received a plus or not. In the "anonymous mode" the sequence is typed, along with its plus or minus, after a variable delay in a way concealing its authorship. In the "standard mode" the sequence typed by each member is not typed on anyone else's teletype;

instead, a sequence from a standard library, stored in the computer, is typed on everyone else's teletype. Each mode is introduced by detailed instructions and considerable practical experience for the family so that they become fully familiar with its distinctive features before beginning the actual problem-solving. Preceding and following each experimental problem each member is given an objective test of his ability to predict the class of sequences that will get a plus; this serves as a test of his ability to recognize relevant patterns.

Progress: In the first study, four families designated as consensus-sensitive were compared with four environment-sensitive families. The distinction between these two groups was made by the use of card-sorting procedure. The consensus-sensitive families were those that sought to achieve consensus at the price of accurate problem solution. The two groups were carefully matched for a number of variables including intelligence and social class. The results were very striking and clear even though, in this pilot study, the sample was small. We used a matched-pair design in which an environment-sensitive family was matched with a consensus-sensitive family. In each pair the consensus-sensitive family showed a progressive improvement in problem-solving ability as intermember access was reduced from the public, to the anonymous to the standard mode. In sharp contrast each environment-sensitive family showed no change or a slight decline in problem-solving ability as intermember access was reduced.

A second study, using a wider sample of 15 families, studied the more detailed processes of hypothesis testing in the two groups of families. Correlational studies suggested that members in families with a marked deficit in the public mode relative to the standard mode, used others' hypotheses as a basis for their own hypothesis testing less often than members in families without such a relative deficit. Moreover, in the public deficit, members were less willing to risk the construction of incorrect hypotheses. These findings were predicted by a consensual experience model: it states that members in environment-sensitive families (with no public deficit) use each others' experiences with the environment as a way of augmenting their own experience and understanding of it, whereas consensus-sensitive families, since they are oriented to achieving an agreed-upon internal version of experience, tend to ignore the others' interaction with the environment. The unwillingness of these individuals to risk negative feedback, for their hypotheses, confirms our view that they experience negative feedback as censorious rather than information-giving; this attitude is consistent with their view of the environment as hostile and unknowable.

After completing the two studies described above, the program for the teletype experiment was substantially revised to: (a) make the three modes more nearly identical in the type and quantity of feedback for subjects' hypotheses and (b) to more completely automate the procedure; this now includes some automatic instruction and testing of subjects. Programming on the SEL has continued throughout the year. Serious delays in completing data collection have been caused by lack of adequate number of research support staff.

Serial No. M-AP(C)-18-6
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Nurse-Doctor-Patient Interaction: An Experimental Study of Its Role in Patient Acculturation on Psychiatric Wards.

Previous Serial Number: M-AP(C)-18-6

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: Richard Almond, M.D., Department of Psychiatry, Stanford University Medical School, Palo Alto, California

Man Years:

Total:	.8
Professional:	.6
Other:	.2

Project Description:

Objectives: The major objective of the study is the direct measurement, under controlled conditions, of nurse-doctor-patient interaction as it occurs on psychiatric wards. The hypotheses of the study are derived from previous work by Dr. Almond which suggest that particular forms of nurse-doctor-patient interaction are crucial to inducing change and improvement in patients. Moreover, his previous studies suggest that certain value orientations by the staff serve to control or perpetuate these crucial interactions and many patients, after modifying their behavior during interaction with professional staff (and other patients), accept these values. This study seeks to relate value orientations of the staff, concerning types and process of treatment as well as personal preferences for types of interaction in group involvement, with the way they interact with each other and with patients. Specifically, we attempt to predict how a nurse and doctor will interact with each other from a knowledge of their value orientations; then we seek to predict how the nurse-doctor dyad will interact with a patient, based on their behavior when interacting as a dyad. This is the first direct experimental study of interpersonal processes amongst psychiatric personnel and therefore the project also serves the general purpose of examining the value of experimental techniques in this area of clinical investigation.

Method: Dr. Almond has modified a questionnaire he used in his original studies in New Haven. It has a broader range of items and is now suitable for administration to nurses and doctors as well as patients. The results of this questionnaire were used to evaluate nurses' and doctors' value orientations. These orientations, in turn, were used to predict nurse-doctor interaction. Two value orientations were selected as independent variables: 1) the subjects' expressed valuation of social openness and involvement in the ward (SOWI) by patients and staff; 2) the subjects' expressed wish to be included in the control groups (FIRO). Sixteen nurse-doctor dyads were formed so that they could be divided into four subsamples of equal size; 8 dyads were highly discrepant on SOWI (more higher) and 8 had similar scores; half of each of these subsamples had highly discrepant FIRO scores (more higher) and half were the same. Each dyad was tested separately in a small-group-type laboratory where they were isolated in booths and could communicate only by microphone. They worked on a standard set of problems, provided by the experimenter, requiring them to group a set of fictitious patients into activity groups. After a series of such problems, they were joined by a patient and continued to work on a set of similar problems as a triad. A number of objective indices of social interaction in the dyads and triads were derived from the card placements and objectively recorded times of various phases of the experimental task. These variables sought to measure the extent to which the dyads and triads 1) agreed or disagreed on problem solutions; 2) blindly followed a potent leader or worked interdependently with each individual making a substantial contribution; 3) utilized the full range of ideas elaborated by each person working alone; 4) "objectified or dimensionalized" the problem rather than seeing each fictitious patient and grouping as unique and personal.

Progress: Data analysis and written reports are complete. The basic findings are these: there is a substantial difference in the effects of independent variables early in the task, when the nurse and doctor are being acquainted and where the dyad meets the patients for the first time, and later in the task when all three have been working together. The findings are these: 1) Where the nurse has a tendency to dominate groups and the doctor wants to be dominated (FIRO compatibility) and the nurse has higher SOWI values, the doctor and nurse form a very tight consensus but are unable to include or assimilate the patient. Here the dominant nurse shows evidence of using her more differentiated values as a club forcing the doctor to accept her views. These findings were also obtained where the nurse and doctor are equal in values and neither is dominating or wishes to be dominated (FIRO incompatible). Here the lack of any differences between the two on value orientation appears to produce an enui exaggerated by incompatibility and papered over by an artificial consensus. 2) Where the nurse and doctor are equal in values and FIRO compatible, and where the nurse is superior in values and the dyad is FIRO incompatible, the nurse and doctor form a flexible and varying consensus and very effectively include the patient in this consensus. In the former, interpersonal compatibility enables the dyad to reach an affable agreement which is well able to include a third person. In the latter, some interpersonal incompatibility focusses the dyad away from the task of simply gratifying one another. Rather they concentrate on preparing for the patient's entrance. The nurse's high SOWI values enable her to take an important role in assimilating the patient when he does arrive. The findings indicate that both independent variables are important and have an interacting effect. A difference in values appears crucial for stimulating the nurse-doctor dyad to consider various approaches and reach a meaningful consensus; however, if the nurse tends to dominate the dyad and the doctor acquiesces, the nurse will use her distinctive value position as a club. Tension between nurse and doctor will be papered over with a forced consensus and the patient will be excluded.

Honors and Awards:

1. Reiss, D.: Invited address. "Assimilating the Patient Stranger: Experimental studies of psychiatrist, nurse, patient interaction." Annual meeting of the Psychiatric Research Society, Washington, D.C., October, 1972.

Publications:

1. Reiss, D., Almond, R., and Shephard, R. "Assimilating the Patient Stranger. Experimental Studies of Psychiatrist, Nurse, Patient Interaction." J. Nerv. Ment. Dis., in press.

2. Reiss, D., "Assimilating the Patient Stranger: Experimental Case Studies of Conflict Resolution Between Psychiatrists and Nurses." Submitted for publication.

Serial No. M-AP(C)-18-10
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: The Effect of Stimulus Materials on Family Problem Solving.

Previous Serial Number: M-AP(C)-18-10

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.1
Professional:	.1
Other:	.0

Project Description:

Objective: In the work done in our laboratory almost all family problem-solving tasks have utilized pattern recognition tasks in which patterned stimuli have been generated by one or another type of finite state grammar (as originally described by Chomsky and Miller). These problem materials have a number of advantages for studying complex perceptual and cognitive processes. Nonetheless, they represent a restricted model of the types of stimulus experience families may encounter in their everyday lives. It is important to establish that our findings concerning family problem solving can be generalized to different kinds of stimuli. Other features of the card-sorting procedure, such as the partial self-administration features, ease of observer recording of sorting performance, uniformity of physical characteristics of the stimulus materials, isolation in booths, have solved a number of long-standing methodological problems. Thus, it seemed imprudent to change the basic card-sorting procedure itself. However, a change of the content of the cards seemed useful to test the generality of our findings.

Method: Accordingly, we designed a procedure, adapted from Tien's Organic Integrity Test, which required subjects to sort cards according to objects pictured on the cards. Three sets of 15 cards were developed: two were used for initial and final individual task and a third for a family task between the individual tasks. Thus, the procedure has the same formal structure as the letter syllable and number-sorting tasks. In each of three tasks the cards can be grouped in at least three ways: by color of object, use of object (e.g. tool, wearing apparel or sporting equipment) or by sex of user (male, female or neutral). A maximally effective sort would presumably use one of these three with complete consistency (corresponding to recognizing the finite state grammar in the basic sorting procedure). Also, families could vary in the amount of sorting similarity between members and across trials. Therefore, the same variables of problem-solving ability, coordination and closure were obtainable from this procedure as the basic procedure.

Progress: Data collection is continuing.

Serial No. M-AP(C)-18-12
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Family Views of Its Social Environment: Effects on Family Therapy Process.

Previous Serial Number: M-AP(C)-18-12

Principal Investigator: David Reiss, M.D.

Other Investigators: Ronald Costell, M.D. and Loann Drake

Cooperating Units: Section on Personality Development, APB, IR, NIMH and Psychiatric Institute of the District of Columbia

Man Years:

Total:	2.0
Professional:	1
Other:	1

Project Description:

Objective: During the past 6 years a series of laboratory studies have revealed that families--functioning as a group--develop shared and vivid concepts of the structure of the larger social community in which they live and work. Some families see their social environment as being patterned and engrossing. Other families see their social environment as threatening, chaotic and overwhelming. Evidence suggests that these shared family constructions of their environment are typical and enduring and dominate a family's response to any novel or stressful challenge in their social environment. We hypothesize that this is particularly true when a family must involve itself in an entirely new social community. Then, their typical reaction patterns play a major role in shaping their adaptations to and involvement in the institution's social processes. This kind of encounter between family and the new institution is very well exemplified by a psychiatric hospital that emphasizes family treatment. When the index member is admitted to the hospital, the whole family is actively engaged in the treatment program. Thus, the family--as a group--must orient itself to a set of novel and stressful challenges. This study is designed to test the relationship between family reaction patterns, as measured in the laboratory, and their adaptation during the first six weeks of family-oriented in-patient psychiatric treatment. The laboratory measures will be used to predict the degree to which the family will become involved and engrossed in the treatment program or withdraw and protect itself from the hospital community and its programs. The interaction between family and hospital will also be viewed as protypical of interactions between families and a variety of institutions in their social environment. Later, we plan studies of the interaction between families and schools and families and occupational settings.

Method: Our independent variable is the family's problem-solving behavior in the laboratory. Based on this, a family can be placed in one of three categories: environment-sensitive, consensus-sensitive and distance-sensitive.

The dependent variables are selected to measure various aspects of the family's adaptation to the hospital community and its treatment program. Two procedures estimate the family's shared experience of the ward.

One procedure is the Ward Perception Q-Sort. Our theory states that a family's typical approach to construing novel social situations will determine its shared experience of the ward community. This shared experience is a variable intervening between family type and adjustment to the treatment program. The Ward Perception Q-Sort is intended as a direct estimate of this shared perception in the family. Specifically, it measures four aspects of the family's shared perceptions: accuracy, stereotypy, coherence and the similarity between members. The procedure requires family members, each individual in his own booth, to sort a group of 36 cards into 7 categories. Each card contains a description that might be applicable to the psychiatric ward and the 7 categories are labeled in descending order from "most characteristic" to "least characteristic." The family is urged to discuss together, through an intercom, how to categorize the cards. Objective scores are computed to estimate the four aspects of family perception from their sorts.

A second procedure is the Family Perception Procedure. This procedure is aimed at assessing, from a different perspective, the character of the family's experience of the social community--in this sense supplementing the Ward Perception Q-Sort. Here, a family is tested immediately following a multiple-family group meeting. Members, working together, are asked to make a number of distinctions between different families and between different members of the same family. Following general procedures outlined by George Kelly, an estimate is made of the number, complexity and character of the dimensions the family uses to discriminate between individuals and families within the group. Here, we attempt to distinguish between simple, moralistic and superficial distinctions and complex, flexible and psychologically insightful distinctions.

Three other procedures permit a direct assessment of family-hospital interaction; they are utilized directly on the ward. Both depend on the family's participation in a multiple-family therapy group--a major treatment modality at both the Psychiatric Institute and NIMH.

The first procedure is direct observation and measurement of the family's interaction with other family and psychiatric staff in the group. Over a series of sessions the family's seating arrangement and the frequency and direction of their speech will be carefully recorded by an observer. The family's engagement and involvement in the ward community will be indexed by its willingness to disperse itself physically in a group rather than stay huddled together in adjoining seats; it will also be indexed by frequent speech directed at many individuals.

The second procedure is the sociometric technique. It, too, is being used as a measure of family engagement and involvement. In particular, we are interested in the experiential boundary the family constructs between itself and the rest of the community. Family members are asked to choose others in the multiple family group whom they like and know well. Impermeable boundaries will be indicated by families who choose few others and where members choose others in the same family. Members in families with impermeable boundaries should also be the object to choice by few others, outside the family, in the multiple family group.

A third procedure is a group cohesiveness questionnaire adapted from studies of outcome and process in therapy groups of unrelated members. This is used to estimate the family's subjective sense of being involved in the multiple family group.

Overall, we have made the following predictions. Environment-sensitive families--who can develop progressively more complex, subtle and accurate solutions to laboratory problems--will show simple kinds of evaluations of the ward social community on the Q-Sort and Family Perception Procedure. They will also show evidence of greater engagement and involvement in the therapeutic process. Consensus-sensitive families--who force an early agreement among themselves on simple and stereotyped solutions to laboratory puzzles--will show similar characteristics in their conceptions of the social community on the ward and remain isolated and distant from the therapeutic program.

Progress: Although some progress has been made in this project during the past year, our work has been characterized more by frustration than accomplishment. The major indecision concerning the future of the Branch and the inability of the IRP personnel office to provide adequate attention to hiring supporting staff, already authorized by the program, has made it impossible to set up a regular data collection schedule with our cooperating institute, the Psychiatric Institute of D.C. The uncertainty about the future of 3 West has brought data collection there to a complete halt after only three families were tested. Indeed, this study--the most promising and significant venture ever undertaken by our Section--has probably been irreparably damaged. One must sadly note that the planning and pilot phases consumed many person-years of time and a considerable financial investment by the program.

Serial No. M-AP(C)-18-13
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Values and Atmosphere on a Psychiatric Ward: Basic Dimensions and Institution Comparisons.

Previous Serial Number: M-AP(C)-18-13

Principal Investigator: Ronald Costell, M.D.

Other Investigators: David Reiss, M.D.

Cooperating Units: NIH Nursing Administration, Nursing Units 3 West, 3 East, 4 West, 4 East, and Psychiatric Institute of the District of Columbia

Man Years:

Total:	.9
Professional:	.4
Other:	.5

Project Description:

Objective: Studies of psychiatric wards indicate that the attitudes and values of staff and patients with regard to therapy-related behaviors play an important role in defining the therapeutic milieu and the nature of therapeutic transactions between patients and treatment staff. This study seeks to explore the relation between therapeutic milieu attitudes and values and aspects of individual disposition toward interpersonal relations. In addition, the study seeks to explore the relation between these two domains and the ways in which individuals perceive the ward atmosphere. These relations are explored through analysis of a composite paper and pencil instrument. Therapeutic milieu attitudes and values are represented on the questionnaire by several previously studied scales including the Almond Social Openness-Ward Involvement Scale, and the Therapeutic Ideology Scale of Strauss. Individual interpersonal disposition items are represented by the A-B Therapist Scale of Whitehorn and Betz, the FIRO-B scales of Schutz and the Machiavellianism Scale of Geist and Christe. The Ward Atmosphere Scale of Moos provides the measure of perception of the social atmosphere of the ward. Exploration of therapeutic milieu attitudes and values, and of interpersonal relations disposition in conjunction with another study, in 1969, provided interesting factor analytic factor clusters from two institutions. In the current study we plan to assess the reliability of these factors over time and extend the perspective of the study to perceptions of ward atmosphere.

Method: The instrument was administered to inpatients, nursing staff and professional staff on the four psychiatric units at NIMH and to similar groups at a large private psychiatric hospital, the Psychiatric Institute of D.C. Individuals from both hospitals participated in the 1969 study. The data consisting on score values of the various component scales will be factor analyzed using the principal components method. Reliability of the questionnaire components and factor structure obtained in the 1969 study will also be assessed. The total sample of respondents will be about 250.

Progress: Questionnaire materials allowing for direct IBM card punching from the forms, and computer program from extracting the scale scores have been prepared and pretested. All questionnaires have been returned, responses coded and punched and primary data analysis well underway.

Serial No. M-AP(C) 19-1
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Progress Report
July 1, 1972 through June 30, 1973

Project Title: Comparative Studies of Discordant Siblings in Families of Schizophrenic, Juvenile Delinquent and Well-adjusted Young Adults

Previous Serial Number: same

Principal Investigator: William Pollin, M.D.

Other Investigators: none

Man Years: none

This project was temporarily suspended and is being held in abeyance.

Honors and Awards: none

Publications: none

Serial No. M-AP(C) 19-2
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies in the Development of Personality and Psychopathology in Identical Twins Discordant for Schizophrenia

Previous Serial Number: same

Principal Investigator: William Pollin, M.D. and Richard Wyatt, M.D.

Other Investigators: Robert Belmaker, M.D.

Cooperating Units:

A. NIMH:

Laboratory of Clinical Science (Dr. Dennis Murphy)
Laboratory of Clinical Psychopharmacology

Man Years (computed for the 12 month period)

Total:	3.1
Professional:	1.2
Other:	1.9

Project Description:

Objectives:

In identical twins discordant for schizophrenia, genetic, racial, ethnic, social class and a variety of other variables which have seemed to bear some relationship to the pathogenesis of this illness are constant for both twins. Therefore, in studying pairs in which one twin is schizophrenic and the other is not, there exists an unusually favorable situation within which to attempt to determine what life history events, family relationship patterns and non-genetic biologic factors play a role in the etiology of this illness, and in what manner. In addition, a new method of analyzing and comparing twin and control data has made it possible to define probable genetic factors which predispose to schizophrenia. The overall picture of schizophrenia pathogenesis which is then possible enables one to aim at a more rational approach to development of new treatment modalities.

Methods Employed:

Previous reports have described the intensive multidisciplinary inpatient work-up of a total of 25 families with identical twins admitted for evaluation to the Clinical Center. The methods used during the past year are the same as those used previously.

Patient Material:

See previous reports for details of the twin population. The 23 normal controls were volunteers who had never had a psychotic illness. The average age of the controls did not differ from that of the patients and bloods were drawn and processed under identical circumstances.

Major Findings:

During the past year the monozygotic discordant twins have been utilized primarily to determine whether certain enzyme abnormalities found in the blood platelets of schizophrenics were genetically determined. In addition, a follow-up study of the twins has been completed.

It had previously been found in a group of 33 patients with chronic and acute schizophrenia that platelet monoamine oxidase activity was reduced. In that study and subsequently, attempts were made to determine how influences secondary to the process of being schizophrenic, such as diet and drugs, as well as hospitalization might have affected platelet monoamine oxidase. It was hypothesized that if the schizophrenic and his non-schizophrenic co-twin had low monoamine oxidase levels, the enzyme abnormality would most likely be related to genetically determined factors rather than being an artifact. In the present study 13 pairs of monozygotic twins discordant for schizophrenia and 23 normal non-twin controls were studied. The blood of each patient was drawn either in his home or in a nearby hospital where platelets were prepared and coded prior to their transport back to Bethesda.

Platelet monoamine oxidase activity was determined by measuring the deamination of 14-C tryptamine bisuccinate, and expressed as the amount of 14-C labelled metabolites formed per milligram platelet protein per hour. The mean ± Standard Deviation (6.4 ± 2.7) enzyme activity of the 23 normals was no different from that of 22 normals previously studied. The schizophrenic twins' mean monoamine oxidase activity (3.9 ± 3) was significantly lower (t test; $p<0.005$) than that of the 23 normals. The non-schizophrenic twin group values (4.7 ± 2.9) were also lower ($p<0.05$). There was no significant difference in MAO activity between the schizophrenic and non-schizophrenic twins. There was, however, a significant Pearson correlation ($r=0.67$; $p<0.01$) between the schizophrenic and non-schizophrenic twins' enzyme activity.

The correlation for platelet monoamine oxidase activities between index twins and their non-schizophrenic co-twins indicates that the low monoamine oxidase activities are not solely secondary to being schizophrenic but may be genetically determined. Other possibilities are currently under study.

In a previous study we had determined that an enzyme capable of forming the hallucinogin dimethyltryptamine (DMT) was present in human blood and that the activity of this enzyme seems to be elevated when measured in nondialyzed platelets of psychotic patients. Blood samples obtained at the same time as those for the platelet monoamine oxidase were examined for the activity of this enzyme by incubating N-monomethyltryptamine with C^{-14} S-adensylmethionine. Results of the assay were described in counts per minute per milligram of platelet protein. The 22 normal subjects' mean ± S.D. enzyme activity was 123.5 ± 55 counts per minute; similar to that described for the original group of normals. The mean enzyme activity for the schizophrenic twins was 190 ± 164. The schizophrenic twins' enzyme activity was significantly higher ($p<0.05$) than the normal controls, but not significantly different than the non-schizophrenic co-twins. A Pearson Product Moment correlation between the schizophrenic and non-schizophrenic twins' platelet enzyme activity was not significant ($r = -0.40$). The results of this study seem to indicate that the nondialyzed platelet enzyme capable of forming dimethyltryptamine is not elevated in psychotic patients because of genetic factors. It appears that the enzyme elevation is either due to an environmental cause prior to the subject's becoming psychotic, or is in fact secondary to the psychosis itself.

At the time of blood drawings of the patients described above, each patient was interviewed extensively by one of us to determine any changes in the patient's life or mental status that occurred since the preceding interview. Sixteen of seventeen pairs that had previously been seen at the NIMH were reviewed. One of the sixteen pairs had definitely become concordant while in three pairs the control twin was now showing possible but inconclusive signs of schizophrenic breakdown. The follow-up time was 7.8 years indicating that the minimal concordance rate in this group was 6.25% while if the three possible cases were considered, it might go up to as high as 25%.

Significance to Mental Health Research:

Previous analysis has focused on the significance of defining a) non-genetic constitutional contributions toward the pathogenesis of schizophrenia, and b) a characteristic pre-illness life course preceding schizophrenia. The findings of the past two years suggest some of the genetically determined biological factors which contribute to predisposition to this psychosis.

Proposed Course of Project:

Continuing analysis of multidisciplinary data with further efforts to trace central relationships are proposed. New biochemical techniques as they become available will be used, as well as further examination of the substrates and inheritance of the enzyme abnormalities for monoamine oxidase will be pursued.

Honors and Awards

Dr. Wyatt presented talks on the above materials to the following: Northern Virginia Community and Mental Health Center, Falls Church, Va.; George Washington University Medical School, Department of Pharmacology, Washington, D.C.; Rockland State Hospital, Orangeburg, N. Y.; Duke University Medical School, Durham, N.C.; Johns Hopkins Medical School, Department of Psychiatry, Baltimore, Md.; and Howard University, Department of Psychology, Washington, D.C.

Publications:

Wyatt, R.J., Murphy, D.L., Belmaker, R., Cohen, S., Donnelly, C., Pollin, W.: Reduced platelet monoamine oxidase activity: A possible marker for vulnerability to schizophrenia. Science 179: 916, 1973.

Serial No. M-AP(C) 19-3
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Coronary Prone Behavior Pattern in the NHLI Type II Study

Previous Serial Number: none

Principal Investigator: Robert Belmaker, M.D.

Other Investigators: William Pollin

Cooperating Units: NHLI Type II Coronary Intervention Study
NHLI, Molecular Diseases Branch
NHLI, Cardiology Branch
Dr. Ray Rosenman, Harold Brunn Institute for Cardiovascular Research, San Francisco, Calif.

Man Years (computed for the 12 month period)

Total:	.3
Professional:	.1
Other:	.2

Project Description:

Objectives:

Studies in various centers have suggested a strong role for behavior as a factor in the pathogenesis of coronary artery disease. Specifically, a coronary-prone behavior profile has been developed that consists of haste, impatience, time pressure, and tenseness. The National Heart and Lung Institute is engaged in a large-scale study of patients referred with hypercholesterolemia. Some of these patients are determined to have coronary artery disease and others are determined to be free of coronary artery disease. A sub-sample of those with coronary artery disease are being followed prospectively by the National Heart and Lung Institute and evaluated with arteriograms for progression of coronary artery disease. The objective of our study is to determine if there are any differences in behavior patterns between those patients who have hypercholesterolemia, but do not have coronary artery disease, and those patients who have both hypercholesterolemia and coronary artery disease. In addition, an objective of this study will be to determine if behavior pattern affects the progression of coronary artery disease as determined by arteriograms. Thus, our study will determine whether behavior pattern is an important risk factor for coronary artery disease even in those patients already predisposed with hypercholesterolemia.

Methods Employed:

The patients are referred from the Type II Coronary Intervention Project and are given a 15-minute standard interview by Dr. Belmaker. This interview is videotaped. In addition they are asked to complete the Adjective Check List and the Jenkins Activity Scale which have been shown to correlate with coronary-prone behavior pattern. Dr. Belmaker is, of course, blind as to whether the patient has coronary artery disease or not.

Patient Material:

See above

Major Findings:

None

Significance to Mental Health Research:

Much effort has been expended to try to unravel the causes of our present national epidemic of coronary artery disease. If life style and behavior pattern are contributing factors, we need to know this in order to reduce incidence of the disease.

Proposed Course of Project:

Presently two to five patients are interviewed weekly and plans are to continue through July, 1974. At that time an estimated sample size of approximately 250 patients will allow sophisticated statistical analysis of relationships between behavior pattern and coronary artery disease.

Honors and Awards

None

Publications

None

Serial No. M-AP(C) 19-5
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Progress Report
July 1, 1972 through June 30, 1973

Project Title: The Distribution and Concomitants of Schizophrenia, and Other Psychopathologies, in a Systematic Sample of 15,909 Twin Pairs

Previous Serial Number: same

Principal Investigator: William Pollin, M.D.

Other Investigators: none

Man Years: none

This project has been temporarily suspended and is being held in abeyance.

Honors and Awards: none

Publications:

Allen, M.G. and Pollin, W. Schizophrenia in twins and the diffuse ego boundary hypothesis. In Cancro, R. (ed.). Annual Review of the Etiology of Schizophrenic Syndrome. New York, Brunner/Mazel, pp. 285-293, 1972.

Serial No. M-AP(C) 19-6
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Twin Intrapair-Comparative Technique in the Study of the Determinants of Early Personality Development

Previous Serial Number: same

Principal Investigators: Mrs. Eleanor Dibble and William Pollin, M.D.

Other Investigators: none

Cooperating Units:

Section on Scientific Applications, AMCS, NIMH, Messrs. Nils Mattsson, Robert Rawlings, Jr., Darryl E. Bertolucci
Social Work Section, NIMH
Child Research Branch, NIMH (Nursery School), Dr. Charles Halverson and Gail Inoff (M-CR-24 (C))
Department of Psychiatry, Georgetown University Medical School, Washington, D.C., Drs. Martin Allen and Mary Riedy
Yale University Child Study Center, New Haven, Conn., Dr. Donald J. Cohen
Adelphi Child Study Center, Dr. Stanley Greenspan

Man Years (computed for the 12 month period)

Total:	1.4
Professional:	1.0
Other:	.4

Project Description:

Objectives:

1. To develop methodologies for the study of the antecedents of personality in children, based especially on the comparative study of pairs of twins. To study differences between children in relation to a) non-genetic biological differences between the twins, i.e. differences in the congenital endowment of the children and early differences during the first weeks of life; b) parental personalities and behaviors, specifically, differences in relationship and perceptions of parents to Twin A versus Twin B; c) the impact of such differences in parent-child relationship, and of traumatic experiences such as illness or surgery, on the child; and d) the origins of, and differences between the children in relation to behavior problems.

2. To study the developmental roots of personality and family characteristics which have been related in our other studies on schizophrenia and psychopathology to the origins of serious mental illness in twins.

Methods Employed:

(1) Longitudinal Study. Families of ten sets of twins who had been referred by local obstetricians have been followed since the twin pregnancy was diagnosed. The children's current ages in this cohort range from 3 to 6 years. During the years the children and their families have been followed, a protocol of testing has been accomplished: developmental evaluation and Bayley testing; neurological evaluation; psychiatric diagnostic evaluations of the twins and parents; periodic family interviews; information from the Experimental Nursery School of the NIMH in which the children were studied for a half-day at age 3; evoked cortical potentials; projective psychological testing; and social work evaluations. A procedure is planned whereby Dr. Martin Allen will interview the parents and Dr. Stanley Greenspan, child psychiatrist, will interview the children at age five to six years to study the increasingly complex structuralization of their behavior as they develop more complex coping and defense systems and the fantasies. Special psychological testing including: a Rorschach test, a specially devised twin TAT, the "World Test" for children, the Stanford-Binet (IQ) test and the Bender-Gestalt test, in order to gain information about the twins' psychic development (both conscious and unconscious).

(2) Epidemiological Study. On the basis of our studies on psychopathology and on the longitudinal study of normal twin development, a new study titled "Childhood Personality Development: Twins and the Antecedents of Personality," approved by the Technical Review Committee on March 31, 1972, was begun. This epidemiological investigation aims at exploring the hypotheses and conclusions from the previous studies and extends the size of the sample, explicates and operationally defines particular variables that have emerged from clinical observations, and uses newly devised psychological instruments. These instruments have been described elsewhere (1972 Annual Report).

Families were recruited to participate in this study by personal appearance at local Mothers of Twins Clubs and at the Annual Convention of the National Organization of Mothers of Twins Clubs. Over 700 sets of questionnaires have been mailed out to families throughout the United States who have expressed interest in participating.

Patient Material:

The Longitudinal Study consists of a cohort of 10 sets of twins, 8 monozygotic and 2 dizygotic. The cohort of the epidemiological study currently consists of 309 sets of twins and is expected to go to between 400 to 600 sets as recommended by the Technical Review Committee.

Major Findings:

A. Longitudinal Study. During fiscal year 1973 the following has been accomplished: 1) home visits to all the families which included interviews with both parents and administration of the Caldwell Preschool test to all children. 2) all parents completed a newly devised form, Behavior Problems, on the children and the data is in the process of being analyzed. 3) several sets of the older children (5 or 6 years) have been seen and tested according to the new procedure described above.

B. Epidemiological Study: During fiscal year 1973 the principal effort on this study has been to recruit and gather data. Data from the first 216 families is now in the process of analysis. Early analysis indicates the two newly devised scales, the Childhood Personality Scale and the Parent Report, are reliable in picking up subtle behavior differences between the twins and to reflect differences in the perceptions of fathers and mothers of their own behavior with a particular child. The remaining five questionnaires of the set still need to be tested, but indications are that the study will make a real contribution in relating child behavior to child-rearing behaviors of parents thus adding to knowledge of personality development or where differences in development arise.

Responsibility for the work on the Epidemiological Study is carried jointly with Dr. Donald Cohen, Yale University Child Study Center. Future reports will give details of the analyses.

Both the Longitudinal Study and the Epidemiological Study of twins will provide a testing ground for our hypotheses about genetic and non-genetic antecedents of personality. The psychological instruments which have been developed for the epidemiological study will have broad applicability in other studies which aim at understanding the social and emotional development of children during the first years of life.

Proposed Course of the Study:

See above.

Significance to Mental Health Research:

The Longitudinal Study children are now entering the Oedipal and latency stages of development and offer the opportunity to observe the increasingly complex structuralization of their behavior as they develop more complex coping and defense systems. The Epidemiological Study provides a major testing ground of many of our hypotheses about genetic and non-genetic antecedents of personality with particular relation to our interest in the first months of life, family perception and the development of competence. The questionnaires developed for this study should have broad application in other studies which aim at understanding the social and emotional development of children during the first years of life.

Honors and Awards:

Dibble, Mrs. Eleanor. Guest speaker. Competence and Vulnerability: Studies of the Early Childhood of Twins. Annual meeting of the American Academy of Child Psychiatry. October 13, 1973. New Orleans, La.

Dibble, Mrs. Eleanor: Guest speaker. A Study of Personality and Vulnerability Determinants in Twins. Annual meeting of the Public Health Service Commissioned Officers, May 9-12, 1973, Phoenix, Ariz.

Publications:

Cohen, ., Allen, M., Pollin, W. et al.: Personality development in twins: Competence in the newborn and preschool periods. J. Amer. Acad. Child Psychiat. VII (4): 625-644, 1972.

Allen, ., Pollin, W. and Hoffer, A. Parental, birth and infancy factors in infant twin development. In Chess, S. and Thomas, A. (eds.): *Annual Progress in Child Psychiatry and Child Development*, New York, Brunner/Mazel, Part I, Chapter 2, 1972.

Serial Number: M-AP(C)-20-1
1. Adult Psychiatry Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Recognizing and Interpreting: A Differentiation of Perceptual and Cognitive Patterns

Previous Serial Number: Same

Principal Investigator(s): Winfield H. Scott, Ph.D.

Other Investigator(s): Monte Buchsbaum, M.D.

Cooperating Units: Laboratory of Psychology, Division of Computer Research and Technology

Man Years: (1972-1973)
Total: .20
Professional: .10
Other: .10

Project Description:

Objectives: The purpose of the research is to study contrasting approaches to Rorschach responding, as a way of studying individual differences in thinking and perceiving, to study the relationships between these contrasting approaches and contrasting varieties of symptom formation in psychiatric patients and differing patterns of performances on laboratory measures of perceptual and cognitive variables among psychiatric patients and normal subjects. In earlier work, two different approaches to Rorschach responding were identified. The first, the "Recognitory" approach, is one in which subjects tend to respond to qualities of blots with associations to things previously seen. In the contrasting "Interpretive" approach, subjects attribute qualities to the blots which are not innate in them, qualities such as movement and depth, and these become important determinents of responses. Both approaches can be observed among normal subjects as well as psychiatric patients. The objectives of continuing work on the project are to refine definitions of the contrasting approaches, to study their relationships to clinical symptomatology in psychiatric patients and to laboratory measures of perceptual and cognitive variables among both normal subjects and subjects who are psychiatrically impaired.

Methods Employed: Normal subjects and psychiatric patients are administered the Rorschach in a strictly routinized fashion. In addition, they are administered other psychological tests, including the Wechsler Adult Intelligence Scale and measures of a variety of perceptual and cognitive variables. A scoring system has been developed for interpretive and recognitory features on the Rorschach. Thus statistical analyses are possible to do on the relationship between approaches to Rorschach responding and performances on the laboratory measures of perceptual and cognitive variables.

Major Findings: Factor analyses have been carried out on the associations of the interpretive and recognitory features in the Rorschach protocols of 20 college students, who were normal volunteers. While the recognitory features have emerged in a single factor, the interpretive features are divided into two different factors, which appear to reflect different styles of thinking and perceiving. The component elements of the first interpretive factor suggests that subjects with high scores on this factor tend to think and perceive in terms of ongoing processes, movement and change. In contrast, it suggested that subjects with high scores on the second interpretive factor tend to objectify processes, to think in terms of fixed outcomes of actions, and to think of objects in terms of their uses. Subjects with high scores on the recognitory factor think and perceive heavily under the influence of concrete features of external stimuli, even to the point of stimulus-boundedness. A few relationships have been established among these normal subjects between interpretive tendencies and performances on some laboratory measures of perceptual and cognitive variables. Subjects who are given high global ratings on interpretiveness also have high scores on an Embedded Figures test, and on a size estimation test. They have lower scores on the Category Width test. Interpretive tendencies appear to be associated with field independence.

Significance to Mental Health Research: The research may allow development of new systems of classifying patients in terms of diagnostic, therapeutic and prognostic variables which are associated with fundamental aspects of psychophysiological functioning.

Proposed Course of Project: The project has been written up for publication, and is now terminated.

Honors and Awards: None

Publications:

Ulatowska, Hanna and Scott, Winfield H. Linguistic indicators of perceptual style. Linguistics, in press.

Scott, Winfield H. Measures of Perceptual and Cognitive Tendencies in Psychotherapy Research. In Waskow, Irene (ed.) The Evaluation of Psychotherapy Outcome, in preparation.

Serial Number: M-AP(C)-20-4
1. Adult Psychiatry Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Consensus Rorschach and Focused Feedback as a Clinical Procedure

Previous Serial Number: Same

Principal Investigator(s): Winfield H. Scott, Ph.D., Elizabeth Sherwood, M.A., Carol A. Langsner

Other Investigator(s): None

Cooperating Units: Section on Personality Development

Man Years: (1972-1973)
Total: 1.00
Professional: .8
Other: .2

Project Description:

Objectives: The main objective of the research is to study the family as a small group, in order to identify salient differentiating features of families which may have effects on the functioning of their individual members. A second purpose is to explore the use of the Consensus Rorschach and video and audio tape feedback as a clinical technique which may be used in the assessment of families. Any family can be regarded as a small group, like other small groups which have tasks to perform, requiring a differentiation of roles. Each family has unique features which are likely to effect the functioning of the family as a whole, and the functioning of its individual members as well. The Consensus Rorschach provides a way of studying these features under conditions which are standardized, but which provide families with an opportunity to express important features of the group freely. The current work also involves exploration of the potential applications of the Consensus Rorschach to clinical assessment. The family are made part of the diagnostic team in that they are asked to review their own performance during "feedback" sessions. In these sessions psychologists lead the family members in a discussion of their performance through the use of videotape or audiotape replay of the Consensus Rorschach procedure. A major objective of the research is to examine the effectiveness of the feedback procedure in changing the performance of family members in a subsequent administration of the Consensus Rorschach.

Methods: Each family admitted to 3-West is asked to participate in the project. Both parents, the index patient and the one sibling closest in age to the patient take part in the Consensus Rorschach. They are required to see how many agreements they can reach about what an inkblot looks like. The procedure is recorded on both videotape and audiotape. Each family is randomly assigned to one of four different "feedback" conditions. Depending upon the condition to which they are assigned, families review their performances on videotape or on audiotape, with or without psychologists present with them. At a third session, the Consensus Rorschach is repeated, as is a paper and pencil test. The procedure produces data bearing on different effects of different feedback conditions, as well as on the differences among families in various aspects of performance on the Consensus Rorschach procedure itself.

Major Findings: The experimental aspects of this work have been brought to a close prematurely, before accumulation of a full sample of cases, because no new families are being admitted to the 3-West program. Writing based on clinical observations is now underway, describing aspects of performance which differentiate families in the test situation, which relate to salient aspects of the family as a small group, and which are likely to have effects on the functioning of individual members of the family. Families who have gone through the procedure - families of psychiatrically impaired adolescent children - are regularly underproductive in the Consensus Rorschach procedure. The reasons for low productivity vary from one family to another. While in some families productivity is low because family members are relentlessly competitive, in other families productivity is low because percepts are not sufficiently differentiated for real agreements to be achieved. Families can be differentiated in terms of their test products; in terms of internal processes; and in terms of the family's relationship to the researchers and the research situation.

Significance to Mental Health Research: The project may result in identification of features of families which are likely to have effects on the functioning of individual members, and of particular importance, on the personality development of individual members. It may also result in a technique useful to apply as a part of the clinical evaluation of families prior to their beginning in family therapy.

Proposed Course of Project: The project will be terminated upon completion of the writing which is now in progress.

Honors and Awards: None

Publications: None

Serial Number: M-AP(C)- 20-5
1. Adult Psychiatry Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychological Studies of Patients with Affective Disorders

Previous Serial Number: Same

Principal Investigator(s): Edward F. Donnelly, Ph.D., Dennis L. Murphy, M.D.

Other Investigator(s): Winfield H. Scott, Ph.D., James K. Dent, Ph.D.

Cooperating Units: The Laboratory of Clinical Science, Section on Psychiatry

Man Years: (1972-1973)
Total: .45
Professional: .4
Other: .05

Project Description:

Objectives: The purpose of the research is to study differences in psychological test response patterns between patients with affective disturbances and other psychiatric patients, and to explore the relationship between test response patterns and a range of clinical and experimental variables among patients with affective disturbances. A major goal is to clarify distinctions between patients with unipolar and bipolar affective disturbances.

Methods: Psychological test productions of patients with a variety of affective disturbances constitute the basic data for differentiation of subgroups. The test data include both objective and projective personality tests, as well as an intelligence scale. Patterns of psychological test data are studied in relation to aspects of premorbid adjustment, course of illness during hospitalization, and adjustment following hospitalization.

Major Findings: Differences have been observed in the test response patterns of patients with unipolar depressions on one hand, and bipolar patients - whether depressed or manic - on the other. On the Rorschach, patients with unipolar depressions develop responses which are dynamically expressive communications while bipolar patients develop responses which are concretely related to the inkblots. During the past year, it has been established that among patients hospitalized for depression, a group of unipolar patients can be differentiated from a group of bipolar patients on the basis of patterns of responses to the MMPI. Moreover, there is a difference between unipolar and bipolar depressed patients in the relationship between scores on a depression scale of the MMPI and ratings for depression on the Bunney-Hamburg scales. While there was a significant relationship between the two for unipolar patients, there is not for bipolar patients. This would suggest that there may be a difference between the subjective experience of depression and outward manifestations of depression in behavior among bipolar patients.

Significance to Mental Health Research: The research may contribute to a refined classification of patients with affective disturbances related to psychological test performances, patterns of perceptual and cognitive functioning, aspects of premorbid functioning and post-morbid adjustment.

Proposed Course of Project: Further analyses will be done on data at hand, and additional data will be accumulated on new patients admitted to the Clinical Center for the treatment of depression.

Honors and Awards: None

Publications:

Donnelly, E.F., Murphy, D.L. Primary Affective Disorder: MMPI differences between unipolar and bipolar depressed subjects. Journal of Clinical Psychology, in press.

Donnelly, E.F., Murphy, D.L. Primary Affective Disorder: delineation of a unipolar depressive subtype. Psychological Reports, in press.

Donnelly, E.F., **Murphy, D.L.** Primary Affective Disorder: social desirability, psychopathology and depression in unipolar and bipolar depressed subjects. Journal of Consulting and Clinical Psychology, in press.

Serial Number: M-AP(C)-20-6
1. Adult Psychiatry Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychological Deficits in Selected Neurological Disorders

Old Title: Psychological Assessment of Deficits in Affective Psychosis, Epilepsy and Schizophrenia

Previous Serial Number: Same

Principal Investigator(s): Edward F. Donnelly, Ph.D., Thomas N. Chase, M.D.

Other Investigator(s): None

Cooperating Units: Laboratory of Clinical Science, NIMH

Man Years: (1972-1973)
Total: .40
Professional: .30
Other: .10

Project Description:

Objectives: The objective of the research is to study patterns of deficit in personality, intellectual, cognitive and perceptual functioning in various neurological and psychiatric disorders, and to study drug-related changes in patterns of deficit.

Methods: Patients with various neurological disorders such as Parkinson's disease, Huntington's disease and Down's syndrome are studied with psychological measures of intellectual, personality and central nervous system functioning. These include the Halstead-Reitan Battery, the MMPI and the Wechsler Adult Intelligence Scale. Behavioral observations are recorded on the Bunney-Hamburg scale.

Major Findings: A study of the possible effects of L-dopa on intellectual and memory functioning reveals that I.Q. increments reported by prior studies can be explained as a function of practice effect, a variable difficult to control in longitudinal studies requiring testing and retesting. A long term follow-up of patients on L-dopa therapy suggests that there are no deleterious effects on intellectual functioning, although there is a slight (and non significant) decline in the scores on the Comprehension subtest of the WAIS for a portion of the patients.

Significance to Mental Health Research: From both clinically and empirically derived data, there have been claims for the improvement in intellectual functioning attributed to treatment with L-dopa. Observations would suggest, however, that the improvement can more likely be attributed to practice effect on the tests than to L-dopa induced effects.

Proposed Course of Project: It is anticipated that during the coming year, patients with a variety of neurological disorders will be studied to establish what aspects of intellectual and personality functioning may be effected by the neurological disorders. In addition, the effects of drugs on these patterns of deficit will be studied.

Honors and Awards: None

Publications:

Chase, T.N., Watanabe, A.M., Brodie, H.K.H. and Donnelly, E.F., Huntington's Chorea: Effect of serotonin depletion. Archives of Neurology, 26: 282-284, 1972.

Donnelly, E.F. Chase, T.N. Intellectual and memory function in parkinsonian and non-parkinsonian patients treated with L-dopa. Diseases of the Nervous System, in press.

Eldridge, R., O'Meara, K., Chase, T.N., Donnelly, E.F., Offspring of consanguineous parents with Huntington's disease. In Barbeau, A., Chase, T.N., Paulson, G.W. (eds.): Huntington's Chorea, Raven Press, N.Y., in press.

Serial No. M-AP(C) - 21-2
1. Adult Psychiatry Branch
2. Section on Perceptual and Cognitive Studies
3. Bethesda, Maryland

PHS - HSMHA - NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Studies of Perceptual and Cognitive "Styles" in Psychiatric and Non-psychiatric Subjects.
Previous Serial Number: Same
Principal Investigator: Monte Buchsbaum, M.D.

Other Investigators: Stephen Landau, M.D.; Thomas Bittker, M.D.; William P. Carpenter, Jr., M.D.; Christian Gillian, M.D.; Frederick Goodwin, M.D.; Dennis Murphy, M.D.; John S. Strauss, M.D.; Paul H. Wender, M.D.; Lyman C. Wynne, M.D., and Theodore P. Zahn, Ph.D.

Cooperating Units: Laboratory of Psychology; Laboratory of Clinical Science; and Laboratory of Psychopharmacology, NIMH.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: To relate individual differences in sensory perception and cognitive functioning in human subjects to psychiatric clinical dimensions and to neurophysiological measures; to develop and implement clinically valuable computerized measures of perceptual and cognitive functions.

Methods Employed: Research in this section focuses on disturbances in perceptual and cognitive behavior in psychiatric patients. Its work is closely coordinated with basic electrophysiological and perceptual research in the Unit on Psychophysiology, Laboratory of Psychology, NIMH.

The finding of perceptual and attentional deficits in a variety of psychiatric patient groups has encouraged the development of theories of psychiatric illness and personality structure based on perceptual style. However, the psychiatric patient's report of his perceptions might reflect poor motivation, poor cooperation, failure to attend to instructions or inability to communicate with the examiner, rather than any actual perceptual difference. For this reason the section has emphasized electrophysiological correlates of perceptual behavior using averaged evoked response techniques which demand relatively little active participation from the subject.

We have previously reported that bipolar affective disorder patients and acting-out adolescents manifested relatively greater rates of increase of visual average evoked response (AER) amplitude to increasing stimulus intensity (augmenting) than did unipolar, or nonparanoid schizophrenic patients, who often showed actual decreases in AER amplitude with increasing stimulus intensity (reducing).

Based on the results of several studies, we have viewed evoked response augmentation as indicative of a tendency to increase the perceived intensity of stimuli, to seek external stimulation, and to be relatively insensitive to extremely weak stimuli. Evoked response reducing, in contrast, is associated with a tendency to decrease the perceived intensity, to avoid exciting external stimulation and to be relatively sensitive to extremely weak stimuli. We have used two strategies to attempt to develop clinically significant diagnostic and prognostic evoked response tools. First, a series of clinical group comparisons to explore the range of variation and to further validate the concept: bipolar affective disorder patients actively incorporating sensory stimuli compared with schizophrenic withdrawal; hyperactive and acting out children compared with normal children and adults; narcotic addicts because opiates, which modify response to painful or intense stimulation,are selected by them as the drug of abuse. Second, we have done a series of neurophysiological studies in normal adult volunteers to attempt to understand the pertinent mechanisms which produce individual differences in stimulus-intensity response. These studies are reported under individual project report Serial No. M-P-C(C) - 36, Laboratory of Psychology.

The clinical studies reported here all utilize a similar AER recording technique. AER to four intensities of light were recorded from vertex-right ear leads; components with a positive peak at about 100 msec (P100), a negative at 140 msec (N140) and a positive at 200 msec (P200) were measured.

Major Findings:

1. Affective disorders and biogenic amines. The relationship of sex, age of symptom onset and monoamine oxidase (MAO) activity in platelets to the average evoked response was examined in a new series of 63 patients with primary affective disorders. Male and female bipolar patients had relatively great rates of increase in amplitude with increasing stimulus intensity. Male (but not female) unipolar patients demonstrated a decrease in amplitude at high stimulus intensities. Within the unipolar group there was a reversal of the normal sex differences in AER amplitude. The bipolar-unipolar differences were maintained even when the patient groups were approximately matched for depression. The unipolar-bipolar group AER differences have recently been replicated by Dr. Elliot Gershon working with an Israeli patient sample.

Unipolar patients had higher platelet MAO activity than bipolar patients; within the unipolar group the AER amplitude/stimulus intensity slope and MAO activity were correlated -.60. Of especial interest was that, within the unipolar group, patients who had platelet MAO levels characteristic of bipolar patients also had AER characteristics of bipolars.

Thus the identification of more homogeneous patient groups may be achieved by the use of both biochemical and neurophysiological means.

2. Schizophrenic patients. Sixteen unmedicated acute schizophrenics were tested during the second and sixteenth week of their 18-week hospitalization at the NIH Clinical Center. Staff nurses and psychiatrists rated behavior and symptomatology. For comparison, 21 age- and sex-matched normal outpatient volunteers were also tested. A second comparison group consisted of normals in high and low states of muscle tension. Consistent with previous findings in a small group, schizophrenic patients early in hospitalization showed significantly smaller amplitude AER and little amplitude increase with increasing light intensity (reducing) in comparison with both normals and bipolar patients for P100-N140. When the early and late hospitalization periods were compared, schizophrenic patients in general, and especially patients rated as good premorbid on the Phillips scale, showed a small but significant increase in the amplitude-intensity function toward normal. However, this increase represented only a small fraction of the difference between schizophrenics and controls. When the schizophrenics early in hospitalization were compared with normals in states of high muscle tension, their AER amplitude-intensity functions were similar; relaxed normals differed significantly. Clinical ratings for anxiety, restlessness, depression, hallucinations, cognitive disorganization, severity of psychopathology and global illness did not correlate significantly with AER amplitude or slope of the AER amplitude-intensity function for P100 - N140.

In contrast the N140 - P200 peak showed no amplitude or intensity differences between groups; and N140 - P200 amplitudes showed high negative correlations with nearly every rating scale. These data suggest that early AER components (P100 - N140) may reflect relatively enduring neurophysiological differences between patient groups. The later components seem to reflect the changing clinical state of the patient.

3. Twin studies. Twin studies are reported in individual project report Serial No. M-AP(C) - 21- 4.

4. Catecholamines and the average evoked response in primates. The specific inhibitor of catecholamines synthesis, a-methyl-p-tyrosine (AMPT) and amine precursors L-dihydroxyphenylalanine (L-DOPA) and L-dihydroxyphenylserine (DOPS) were administered to study the role of the putative neurotransmitters dopamine (DA) and norepinephrine (NE) in the electrophysiological events represented by the averaged evoked responses (AERs) to sensory stimuli in non-human primates. In a collaborative study with Eugene Redmond, George F. Borge and James W. Maas of Illinois State Psychiatric Institute, four Macaca speciosa, one male and three females, were implanted with permanent cortical electrodes and trained to accept chair restraint. An average evoked response testing procedure essentially identical to that employed with the patient and twin groups mentioned above was used. Extremely consistent and stable evoked response recordings were obtained and similar drug responses were seen in all four monkeys. After the peripheral

decarboxylase inhibitor, MK-485, which leaves brain DA and NE unchanged, L-DOPA produced small but very consistent augmenting, as did DOPS. AMPT caused marked reducing in two monkeys and mild reducing in two. After AMPT, DOPA caused marked amplitude-intensity slope changes in the monkeys who had the greatest AMPT effect. This suggested that L-DOPA effects were greatest when DA and NE were first depleted, and that the measure might reflect this depletion and explain individual differences in L-DOPA response. Similar average evoked response amplitude intensity findings were seen in a group of seven patients at the NIH Clinical Center treated with AMPT orally on a chronic basis for AER components in the same time range.

5. Amphetamine in minimal brain dysfunctioned children. Visual and auditory evoked responses were studied in 24 minimally brain-dysfunctioned (MBD) children and 21 age- and sex-matched normal controls. The MBD children showed AERs characteristic of relatively younger children. MBD children who showed clinical improvement on amphetamine tended to show these immature patterns while off medication to a greater extent than MBD amphetamine non-responders, suggesting that pretreatment AER may be used to predict drug response. Amphetamine-related changes were also different in the responder and non-responder MBD children. The data support the concept of a neurophysiological "maturational lag" in MBD children. We have hypothesized that the hyperactivity commonly seen in MBD children may reflect the search for additional sensory stimulation to counteract a relatively reduced sensory input.

It is of further interest that the same component which is specifically affected in AMPT and DOPA studies in monkeys and affective disorder patients is also the component differentially affected by amphetamine in MBD children.

6. Lithium in hyperactive children. AER results in a small subsample of hyperactive children treated with lithium in a collaborative study with Drs. P. Wender, L. Greenhill, R. Rieder and T. Zahn showed no AER changes after lithium treatment which was generally ineffective clinically.

7. Narcotic addicts. A group of narcotic addicts in a methadone research program in the Veterans Administration Hospital was studied. The research plan had been to study patients on and off methadone treatment on a single blind basis. Opiates, which modify responses to painful and intense stimulation, would be expected to affect evoked responses at high intensities differently from low intensities. We have further hypothesized that users of addictive drugs may differ from a control population in their baseline off drug electrophysiological response to stimuli of various intensities. Tests of prognostic and diagnostic significance might be expected to develop from such a program.

But unfortunately, although 26 narcotic addicts were tested as outpatients, all but two were on methadone treatment. Administrative difficulties prevented the single blind on and off methadone treatment design from being completed with the Veterans Administration Hospital.

Significance to Biomedical Research and the Program of the Institute: This project, in helping to give a neurophysiological basis to psychiatric diagnosis and prognosis, contributes to the effort to understand the mechanisms of psychiatric disease.

Proposed Course of the Project:

1. Affective disorders and biogenic amines. During the next year detailed studies of evoked responses of patients with affective disorders treated with d- and L-amphetamine will be carried out. Of especial interest will be the individual differences in the drug response, and the use of AERs to predict subsequent response to clinical pharmacological treatment. A series of twins with affective disorders is also being studied. Normal twins, already studied by us, are being studied for platelet MAO.

2. Schizophrenia. We will continue to study patients with schizophrenia with some detailed stimulus intensity measures, and to correlate results with clinical ratings and MAO activity.

3. Addicts. We will obtain data on the effect of methadone on patients in collaborative studies with Drs. T. Chase and L. Ng in the Laboratory of Clinical Science. We will also be collaborating with Dr. Arnold Ludwig at the University of Kentucky in evoked response amplitude intensity studies of alcoholic patients prior to and during the administration of doses of alcohol. In addition, Dr. Bert Kopell, Dr. W. T. Roth, and Adolf Pfefferbaum at the Palo Alto Veterans Hospital will be collaborating with the Section on some studies of narcotic addicts on and off methadone.

Honors and Awards: None

Publications:

Buchsbaum, M., King, C. and Henkin, R. I.: Average evoked responses and psychophysical performance in patients with pseudohypoparathyroidism. J. Neurol. Neurosurg. and Psychiat. 35 (2): 270-276, April, 1972.

Silverman, J., Buchsbaum, M. and Stierlin, H.: Sex differences in perceptual differentiation and stimulus intensity control. J. Person. Soc. Psychol. 25 (3): 309-318, 1973.

Greenhill, L., Rieder, R. O., Wender, P. H., Buchsbaum, M. and Zahn, T. P. Lithium in the treatment of hyperactive children. Archives Gen. Psychiat. In press.

Landau, S. G. and Buchsbaum, M. : Average evoked response and muscle tension. Psychonomic Science. In press.

Serial No. M-AP(C) - 21-4
1. Adult Psychiatry Branch
2. Section on Perceptual and Cognitive Studies
3. Bethesda, Maryland

PHS - HSMHA - NIMH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Perceptual and Cognitive Style in Normal Twins.
Previous Serial Number: Same
Principal Investigator: Monte Buchsbaum, M.D.

Other Investigators: Lyman C. Wynne, M.D., Ph.D.
Theodore P. Zahn, Ph.D.
Stephen Landau, M.D.

Cooperating Units: Unit on Psychophysiology, LP - NIMH

Man Years:
Total:
Professional: 1.5
Other: 1.5

Project Description:

Objectives: Evaluation of the heritability and interaction of several sensory, perceptual and cognitive functions in normal volunteer twins.

Methods Employed:

1) Rod and frame measure of field dependence-independence.
2) Measure of scanning: direct monitoring of eye movements during a size estimation task.
3) Auditory and visual cortical evoked response measures designed to evaluate the effect of stimulus intensity, stimulus contrast, evoked response stability and cortical response symmetry.
4) Rorschach inkblot test.
5) Psychiatric background interview.
6) Clinical rating scales (M-AP(C)-14-1).
7) Blood typing for zygosity (M-AP(C)-16-1).
8) Autonomic and psychomotor functioning.
9) WISC IQ test.

Major Findings:

A group of 30 MZ and 30 DZ adult twin pairs (15 male, 15 female in each group) were studied using visual average evoked responses (AER) to four intensities of light and auditory evoked responses. Each subject received both evoked response procedures on two separate occasions to assess technique reliability. Other perceptual tasks including the Rod and

Frame test and Visual Size Estimation were also administered twice; the full scale WAIS and the Zuckerman sensation seeking scale (SSS) were each given once. EEG was recorded between vertex and right ear. Visual AER s were measured from three peaks--a positive, negative and positive sequence of peaks at approximately 100, 140 and 200 msec (termed P100, N140 and P200, respectively). The AER amplitude/stimulus-intensity-function slope was also computed. Auditory AERs were used to assess AER variability within session using variance and split-half correlation measures of variability. All AER measures showed significant ($P < 0.01$) interclass correlation coefficients between MZ twin pairs; DZ twin pairs generally showed low or nonsignificant interclass correlation coefficients between AER measures. MZ interclass correlations for successive latencies and amplitudes decreased (e.g. mean MZ interclass correlations for AER latencies P100, N140 and P200 were 0.74, 0.69, and 0.45 respectively) indicating greater genetic determination for early neurophysiologic events.

Both variance measures (standard deviation and correlations) for the auditory AER showed moderate (0.40) correlations between both MZ and DZ pairs for early time intervals with increased MZ-DZ differences after 200 msec. In order to assess the effects of non-genetic factors, differences in AER within the MZ group were investigated. Using perceptual tasks, and personality variables with both high and low heritability, MZ twins were divided into two groups--those with high and low intra-twin pair differences. Generally, tasks or tests with high interclass correlations for both MZ and DZ groups were effective in explaining intra-twin pair AER differences. For example, for the SSS, the MZ interclass r was 0.70 and the DZ r was 0.59. The MZ twin pair group with below median SSS differences had AER slope interclass correlations of 0.68; the group with above median differences had an interclass correlation of 0.11. In comparison, the total MZ group interclass correlation was 0.51 and for DZ pairs 0.01.

One possible explanation for the high MZ correlations and very low DZ correlations for AER peak amplitudes and latencies may be that the principal peaks of the AER are determined by several genes, the alteration of only one of which changes the configuration drastically. Visual comparison of the remarkable resemblance of AER in MZ twin pairs is consistent with this view. In contrast the lower but more equal MZ and DZ correlations for AER variability factors suggest a more limited genetic influence, with variability perhaps determined by a single gene which many DZ pairs share in common.

The fact that systematically differing MZ twin pair correlations were seen with successive AER peaks, and that amplitude-intensity slope functions also had high interclass correlations in MZ twins, tends to suggest that simple anatomical features of the skull or cortical tissue are not responsible for the strong AER resemblances in MZ twin pairs, but that the functional neurophysiologic organization of the cortex as well must be involved.

Significance to Biomedical Research and the Programs of the Institute:

The concepts of field dependence-independence and stimulus intensity control have been relevant in predicting and explaining individual difference in pain tolerance, sensory isolation, tolerance and response to stimulus overload. Preliminary studies here at the NIMH have revealed that there are familial tendencies in perceptual styles as measured by standard psychophysiologic tasks and cortical evoked responses. One method of further elucidating the heritable nature of perceptual style is to investigate several perceptual functions in groups of monozygotic and dizygotic twins. This will allow us to draw some conclusions as to what aspects of perception are genetically transmitted and which ones are learned or modified by environmental experiences.

Proposed Course of Project:

Data on rod and frame, eye movements, and rating scales currently under analysis. Evoked response and stimulus contrast and cortical response asymmetry data are also in final stages of analysis. Monoamine oxidase levels in this group of twins are currently being obtained by Dr. Dennis Murphy. Emphasis in subsequent data analyses will be on: 1) pinpointing which precise evoked response components have the highest heritability; 2) determining if biochemical measures correlate most highly with either variable showing large MZ and DZ differences in heritability or variables showing low MZ-DZ differences; 3) further assessing non-genetic factors by examining which perceptual variables explain the variance observed between MZ pairs.

Honors and Awards: None.

Publications: None.

Serial No. M-CR-10(c)
1. Child Research Branch
2. Child Behavior
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Relations of Preschool Behavior to Present and Earlier Parent and Child Characteristics

Previous Serial Number: SAME

Principal Investigator: Charles F. Halverson, Jr.

Other Investigators: Mary F. Waldrop

Cooperating Units: Section on Twin and Sibling Studies, Adult Psychiatry Branch, NIMH (M-AP(C)-19-6)

Department of Educational Psychology, State University of New York at Albany

Man Years:

Total: 9.0
Professional: 2.0
Other: 7.0

Project Description:

Objectives:

The general objective of this project is to establish reliable measures of parent and child behavior to serve as criterion data for the larger branch longitudinal program consisting of studies of family development, newborn behavior, and parent-infant behavior. Marriage and parent behavior is studied in this phase, but the focus is on the child's free play, social response, cognition, and persistence in overcoming obstacles.

Methods Employed:

Children ranging in age from two and a half to three years of age attend our research nursery school for one half-day accompanied by their mother, then, in groups of five, for a four-week period, where their behavior is measured by direct observation and rating of free and semi-structured play. Our assessment centers on the following: the nature of solitary play; reactions to strangers and to brief separation; deployment of attention in free play settings indoors and out; social behavior towards peers and caretakers; and the nature of assertive behavior elicited by several contrived experimental barrier situations. Intellectual performance is assessed via several standardized tests as well as from ongoing speech and play. Parental data is obtained in a controlled interaction situation and in various interviews and contacts with the mother. The latter data will be used to assess current relations between parent and child, and, in conjunction with earlier data gathered by other sections of the branch, will also be used to index developmental change in the parents and in the marriage.

Within-stage analyses will be directed toward the stability of play and social behavior across settings and over time. Relations between the various kinds of measures in the different settings will provide material for composite scores summarizing the characteristics of the child and parents.

Major Findings:

Data collection on the main cohort of the longitudinal phase will be completed in July of 1973, at which time data analysis will begin. By that time 25 groups of children will have been through the four-week procedure. The total number of preschool children seen in half-day studies will be approximately 160 and, in the nursery school, approximately 127.

Several reports have been given at scientific meetings and prepared for publication, based on samples studied in preparation for the cohort described above. In order to understand the first of these to be described it is necessary to provide some background information. Previous studies of preschool children in our own and other laboratories have consistently identified active, vigorous, intense, and rapidly-paced play as a major component of nursery school behavior. There have been a number of attempts to avoid difficulties associated with ratings of activity level by mechanically recording bodily motion in play. Many of these techniques, however, have necessitated the use of cumbersome equipment or recording procedures (such as chair stabilimeters, floor squares traversed, etc.) that limit recording to one locale and thus cannot yield information on the generality of activity level.

In our project we have used a simple and reliable activity recorder that can be used in many measurement situations. The activity recorder consists of three small, hand-wound wristwatches, with balance wheels removed, that are modified so that any movement across one dimension results in a movement of the sweep-second hand of the appropriate watch. Each watch records movement in one of the three spatial dimensions. Three watches are placed in a small packet fastened to a child's back during free play.

We have previously reported limited normative data on this form of activity recorder but had not reached a point of precision in application that would warrant comprehensive study. In a paper accepted for publication this year, we reported new findings on the activity level of 57 normal two-and-a-half-year-old children for whom behavioral data were obtained from three reliable and stable sources: first, from the activity recorders fastened to the back of each child's shirt or jacket during outdoor free play; second, cumulative times and frequency counts for specific categories of behavior over the five-week session; and third, daily ratings made by the teachers on a variety of social and play dimensions.

Results indicated that for outdoor play there was strong evidence of validity for the activity recorders. Out of a total of nine categories of observed activity, six were highly and consistently related to the recorder data. Also, the recorder data from outdoor play were highly related to active, vigorous play in the indoor free play situation for the male sample; boys who were motorically active outdoors tended to be the same in indoor play. The outdoor recorder data were significantly related to 9 of the 14 objective observation measures of gross activity indoors. For the females, however, only 3 out of the possible 14 relations were significant. Even though the activity recorders reliably measured the same aspects of outdoor activity for both males and females, males tended to be consistent across the two situations, whereas female play activity outdoors seemed relatively independent of indoor play.

Correlations between the activity recorder data and teachers' ratings of behavior indicate that for both sexes the kind of activity measured by the recorder was extremely salient for the teachers. Almost all teacher ratings for both sexes were related to gross activity. Out of 27 daily ratings, 25 were significantly related to the activity recorder data for males; for females, 21 of 27 correlations were significant. The ratings summarized the areas of vigor and excitability, withdrawal, peer interactions, and teacher contact. To control for the intercorrelation between the ratings, subjects were given three factor scores based on a factor analysis of a larger sample of 120 males and 141 females who had previously participated in the nursery school. As with the individual teacher ratings, the activity recorder data were significantly positively related for both sexes to two of the three orthogonal factors (Participation vs Apathy, and Hyperactivity) and negatively related to the third (Nurturance Seeking from Teachers). These data provide, on the one hand, a valuable validity check for ratings concerned with vigorous, active play and, on the other, the caution that if we were using teachers' ratings alone, no distinctions would have been found between the sexes relative to situational variability in play activity. These data are also in line with the results of earlier factor analytic studies in indicating that one of the most important areas that can be studied in two-and-a-half-year-old children is vigor and intensity of play. This fact has important implications for our forthcoming efforts to relate our data to earlier longitudinal material.

To broaden our understanding of the concept of activity level we have been cooperating with Dr. James B. Victor of the State University of New York at Albany in a study of activity level in grade school children. Data on 120 grade school children from kindergarten to fifth grade is now being collected by Dr. Victor. Activity recorder scores, teacher and peer judgments of child behavior, and a measure of field dependence will be analyzed when data collection is complete. In a preliminary study of first and second grade boys, activity level was related to teachers' ratings on a behavior problem checklist. This paper has been accepted for publication.

In a paper recently presented at the Society for Research in Child Development meetings we reported on the longitudinal and cross-sectional relations of peer orientation. For this analysis we studied 74 children at age two and a half who attended our research nursery school in groups of five for five weeks. At seven and a half years of age, 62 of the 74 (35 boys, 27 girls) participated in a follow-up study. When the children were two and a half, peer behavior was measured by trained observers who timed and counted various categories of peer interactions, and by two teachers who made daily ratings. When the children were seven and a half we measured peer behavior from a new kind of diary that overcomes many previous problems by having the mother record the occurrence and timing of specific events in the course of the child's day. This diary was kept by the mother for her child's activities during the week prior to the day they both came in for the follow-up study. Particular focus was placed on activities with peers.

From the foregoing data it was found that children of both sexes who at two and a half were friendly, involved with their peers, and who were able to cope with aggressive peers, at seven and a half usually became children who spent many hours outside school with peers and were socially at ease. The latter were dominant ones who, when they were with peers, decided whom would be included and what they would play. Social behavior at seven and a half had very different meanings for boys than for girls. The highly social preschool boys later, as early school-age children, tended to have extensive peer relations. That is, they usually played with groups of boys. For boys, the diary record of hours spent with peers correlated .82 with hours spent with more than one peer. The highly social girls, when with peers, tended to have intensive peer relations. That is, they usually played with only one other girl. For girls, hours with peers correlated .86 with hours with only one peer.

Extensiveness for males and intensiveness for females had common significant correlates with two-and-a-half-year-old and seven-and-a-half-year-old behaviors. Measures at two and a half that significantly correlated with extensiveness for males and with intensiveness for females were: friendliness, involvement with peers, coping with peers, coping with object blocks, number of different play activities, originality, time spent looking and listening, not being fearful, and not being withdrawn. Behaviors at seven and a half that significantly correlated with both extensiveness for males and intensiveness for females were: social ease, dominance, importance of peers, number of hours with peers, being with peers of the same sex and same age, scores on the Vineland Social Maturity Scale, and not being fearful.

Cooperative research with the Section on Twins and Sibling Studies of the Adult Psychiatry Branch, NIMH (M-AP(C)-19-6) continued into this year. Using our play rooms, and with some assistance from our personnel, their staff has begun follow-up studies to five and a half years, on 10 sets of twins studied earlier in our preschool program. Data collected in our nursery school has already been related to perinatal material gathered in the twin section, and a report published. Further and more refined analyses of the twin data await completion of major analyses of the larger cohort of nursery school subjects.

Significance to Bio-Medical Research and the Program of the Institute:

The delineation of patterns of social, emotional, and cognitive behavior in the young child will help clarify the course of development during the childhood years. The present project provides valuable documentation of the varieties of adaptive and maladaptive preschool behavior, as well as clues to the origins of these behaviors, stemming from analyses of data collected earlier on these same children and their parents. Mental health planning and services depend on information of the sort uniquely provided by the longitudinal data available to this project.

Honors and Awards: None

Publications:

Halverson, C. F., Waldrop, M. F.: The relations of mechanically recorded activity level to varieties of preschool play behavior. Child Develpm., 1973, in press.

Cohen, D. J., Allen, M. G., Pollin, W., Inoff, G., Werner, M., & Dibble, E.: Personality development in twins--competence in the newborn and preschool periods. J. Amer. Acad. Child Psychiat., 11 (4): 625-644, 1972.

Victor, J. B., Halverson, C. F., Inoff, G., & Buczkowski, H. T.: Objective behavior measures of first and second grade boys' free play and teachers' ratings on a behavior problem checklist. Psychol. in the Schools, 1973, in press.

Serial No. M-CR-11 (c)
1. Child Research Branch
2. Infant Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Relations between Human Neonatal Behavior and Later Development

Previous Serial Number: SAME

Principal Investigator: Raymond K. Yang

Other Investigators: Richard Q. Bell

Cooperating Units: The nursery facilities of 13 Washington Metropolitan Area hospitals

Man Years:

Total:	3.25
Professional:	1.25
Other:	2.00

Project Description:

Objectives:

This is a psychophysiological and behavioral study of human neonates designed to identify congenital characteristics of infants born to families in the Branch Longitudinal Study. The data are used to: 1) identify stable measures of individual differences in human newborns; 2) locate continuities and discontinuities between the newborn period and later periods of development; and, 3) describe the fashion in which newborn characteristics interact with parental variables.

Methods Employed:

The data consist of physiological and behavioral recordings obtained from 138 human neonates between 1967 and 1970. The physiological data consists of continuous recordings during two inter-feeding periods (totalling five hours) of heart and respiratory rate, skin temperature, skin resistance, and electro-oculograms. The behavioral data consists of ratings of spontaneous motor activity during sleep, and sensorimotor measures including responses to tactile, aversive, sucking, and quieting stimuli. Also available are complete hospital records on the delivery of the infant.

All data collection occurred in the hospital where the infant was delivered. Average age of the infant at testing was 60 hours.

Major Findings:

The period following completion of data collection has been devoted to transformation and reduction of the physiological recordings to forms amenable to statistical analyses. This year preparatory analyses have been completed and the test-retest reliabilities of heart and respiratory rate, sleeping patterns and the behavioral measures have been established. Within-stage analyses have been initiated. Cross-stage analyses linking neonatal data to data obtained at other stages in the longitudinal study have not yet begun.

Three within-stage issues are currently under investigation. The first deals with the relationship between drugs (analgesia, anesthesia, oxytoxin) given a mother during labor and the behavior of her infant immediately after birth. Complete records of delivery are available in the Section and attempts are being made to develop a scale representative of drugs administered to the mother with regard to factors such as type of drug, time of administration, and potentiating and/or synergistic effects of drugs given in combination. Preliminary analyses on several subsamples fail to confirm the frequently reported finding of a depressive effect on newborns of drugs administered the mother during labor. The second issue deals with the possible relationship between newborn measures and minor physical anomalies (e.g., single transverse palmar crease, double hair whorls, etc.). Waldrop and Halverson (Section on Child Behavior) have demonstrated a relation in preschoolers between hyperactive behavior and these minor physical anomalies. The anomalies are present at birth and appear to be stable at least to early school age. We are currently investigating the possible relation between these anomalies and basal autonomic rates (sleep patterns, heart and respiratory rate), reactivity to aversive stimulation, and discriminative sucking behavior. Analyses to this date suggest that the anomalies will not be related to single measures, but may possibly be related to combinations of scores. The third issue deals with responses to threshold tactile stimulation, and is a continuation of analyses begun in 1972. Current analyses of patterns of heart rate changes to threshold and prethreshold stimuli suggest that cardiac-somatic coupling exists in newborns.

A further study is underway of data obtained from a four-month-old anencephalic infant whose general responses to various stimuli have already been reported in the psychological literature. Preliminary analyses of behavioral and heart rate responses to repetitive auditory and olfactory stimuli suggest that while differentiated behavioral and heart rate responding occurs, no orderly patterns of response can be established that can be attributed to the repetitive stimulations, (as have been found in normal infants).

A study has been published reporting that normal newborns, when placed prone rather than supine in their isolettes, sleep more, cry less, and move less. This suggests that when quiescence is desirable, prone placement is more effective in maintaining this state than supine placement.

Significance to Biomedical Research and the Program of the Institute:

Results from an earlier publication (Bell, Weller and Waldrop, Monogr. of the Soc. for Res. in Child Develpm., 1971, Vol. 36, Nos. 1-2) suggest that paradoxical shifts in relations may occur between measures obtained during the newborn period and during the preschool period (2-1/2 to 3 years): apparently prognostically healthy signs at the newborn period (vigorous respiration and reflex responsiveness) were positively related to watching others rather than participation behavior at the nursery school period. In a symposium at which these findings were presented, a discussant noted that a similar relationship existed in data from the Fels and Berkeley longitudinal studies. Although these studies lacked newborn data there seemed to be some indication that paradoxical shifts such as we had found (early high intensity behavior associated with later low intensity) had occurred where the early measures had been obtained between six and fifteen months. Last year we located two follow-up studies from the newborn period, one to four months, the other to eight months, in which the data show the same shift. It is possible there may be at least two paradoxical inversions of intensity occurring in the first year, one occurring before the fourth month, the other before fifteen months. We are planning to examine this possibility in Cohort II data. Extensive physiological and sleep data were not available in the study by Bell et al.

Proposed Course of the Project:

Within-stage analyses are currently underway. Once these are completed, we plan to analyze and report on relations between newborn measures and later behavior at three- and eleven-months postpartum, the latter obtained by the Parent-Infant Section. Relations between newborn and preschool characteristics cannot be determined until 1975, since data gathering at this later phase will not be completed until 1973, and reduction of the data will not be completed until two years later.

Honors and Awards: NONE

Publications:

Levin, H., Grossman, J., Kaplan, E., & Yang, R. Constraints and the Eye-Voice Span in Right and Left Embedded Sentences. Language and Speech, 1972, 15, 30-39.

Brackbill, Y., Douthitt, T.C., & West, H. Psychophysiologic effects in the neonate of prone versus supine placement. J. of Pediatr., Vol. 82, No. 1, 82-84.

Serial No. M-CR-12 (c)
1. Child Research Branch
2. Section on Family Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Developmental Patterns in the Young Family

Previous Serial Number: SAME

Principal Investigator: Robert G. Ryder

Other Investigators: David H. Olson, John S. Kafka, and Walter Sceery

Man Years:

Total:	6.3
Professional:	2.0
Other:	4.3

Project Description:

Objectives:

To identify and describe variations in relationships in young married couples; to describe and explicate the developmental history of differing patterns of relationships; to integrate subcultural, social, and personality phenomena in a comprehensive view of intimate human relationships; to investigate correspondance among marriage patterns, attributes of offspring, and patterns of parent-child interaction.

Methods Employed:

Most of the data presently being studied were previously collected from a sample of 2,162 young married couples living in the Washington D.C. metropolitan area. Data collection in the period covered by this report has been limited to marriage followup studies of 120 couples whose children have reached the preschool phase of the Branch longitudinal study. Data collection includes interaction procedures, questionnaires and an interview.

Major Findings:

Profile rotation of data from the Color Matching Test based on separate analyses of two samples, consisting of 193 couples each, yielded four dimensions of interaction in married couples. The first of these dimensions is one of "rationality" versus affectivity. Some spouses tend to deal with each other in a flat and unemotional way, and to present a logical and "reasonable" interactional style. Other spouses deal with each other less in terms of reasons, and more in terms of open affect, both negative and positive. This first dimension is a clear replication of results from earlier Color Matching Test analyses, and was shown in earlier analyses to have substantial relationships with marriage data from other sources. The second dimension distinguishes couples who tend to express positive affect in their interaction, from couples who tend to express negative feelings. The third and fourth dimensions seem to reflect some basic patterns with which spouses win in interpersonal disagreements. In one such pattern, for example, the winning spouse is not openly emotional, and is reasonable in manner, while the losing spouse expresses both positive and negative affect. Altogether, scored data for the Color Matching Test exists for approximately 800 couples. Data for 400 of these cases are being witheld from current data processing, to be used in later replication of possible ad hoc analyses.

Data from the other interaction procedure used in this project, the Inventory of Marital Conflict, is to be completely rated and coded by the end of this fiscal year. It will therefore be possible in FY 74 to determine whether similar dimensions of interaction are supported by a second measurement procedure, and whether couples' patterns of interaction are consistent over time.

Several activities which were in process in FY 72 have been completed and reported out in FY 73. A longitudinal analyis dealing with the marriage correlates of having a child is one of these, and is presently in press. The same is true of an impressionistic analysis of young couples in what has been referred to as the counter culture. In considering somewhat the same kind of material, David Olson has ventured some predictions concerning the future of marriage, in another article that is also in press. Basically, Olson suggests that the institution of marriage is changing, but gradually so, in a manner he thinks of as evolutionary rather than revolutionary.

In earlier work, various kinds of individual, family and group therapy were organized by David Olson according to a heuristic system framework. He has now applied the same framework to a classification of various research studies of behavior therapy, so that each is viewed according to the social unit under analysis, and other cognate factors. From this classification, the type of research which has been most slighted by investigators, and which is therefore most in need of a higher priority, becomes readily apparent.

We have described a number of research activities of our staff. However, by far the greatest portion of the effort in this project during FY 73 has been spent in turning the large amount of extant data into manageable and usable form. When each component of the data has reached such a form it should be possible to proceed in an orderly way to a systematic analysis of the data set as a whole. Activities along these lines range from building computer files to constructing special purpose computational tools. Two of the latter, a procedure for cluster analysis, and one for sequence analysis, were described in the FY 72 report. This year, the special purpose programs which have been assembled include procedures for item analysis, factor rotation, and organizing coded interaction data. There are always more details to be taken care of in this kind of task than one expects, but preparatory work is at present largely completed, and the project can now be essentially devoted to substantive cross-sectional and longtudinal analyses.

Significance to Bio-Medical Research and the Program of the Institute:

Those who welcome social change and those who oppose it are joined in perceiving a close link between change in society and change in the basic family unit. Those concerned in one way or another with such topics as social maladjustment, drug use, alcoholism, social activism, feminism or sexual libertarianism, must at some point confront the family patterns or the changes in family patterns that reflect, support, or hinder these various tendencies. There is no way to do this without being able to describe systematically the various marriage and family patterns presently extant, and without having methodology that will adequately reflect development and changes in these patterns.

In the view of many professionals, it is becoming more and more clear that one of the greatest shortcomings of much mental health research has been a failure to take adequately into account the social context in which events occur, including familial, subcultural and other sources of influence. This project has been linked with others in the area of child development in an attempt to remedy this deficiency.

Proposed Course of Project:

Substantive longitudinal and cross-sectional analyses of data presently in hand will be the chief concern in FY 74. Since data is presently becoming available from other components of the Branch, longitudinal analyses relating marriage data to infant and child data will now be possible.

Honors and Awards:

The principal investigator was appointed to the board of directors of the Groves Conference on Marriage and the Family.

Publications:

Kafka, J.S. and Ryder, R.G.: Notes on marriage in the counter culture. Journal of Applied Behavioral Science, in press.

Olson, D.H.: Marriage of the future: Revolutionary or evolutionary change? The Family Coordinator, 21: 383-393, 1972.

Olson, D.H.: Review and critique of behavior modification research with couples and families, or: Are frequency counts all that really counts? In Richard Rubin (Ed.), Advances in Behavior Therapy, in press.

Olson, D.H. and Straus, M.A.: A diagnostic tool for marital and family therapy: The SIMFAM technique. The Family Coordinator, 21: 251-258, 1972.

Ryder, R.G.: Describing variations among marriages. In Jacob Christ and Henry Grunebaum (Eds.), Marriage: Problems and Prospects, in press.

Ryder, R.G.: The relationship between having a child and changes in reported "marriage satisfaction." Journal of Marriage and the Family, in press.

Serial No. M-CR-22 (c)
1. Child Research Branch
2. Infant Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Offspring Effects on Parents

Previous Serial Number: Same

Principal Investigator: Richard Q. Bell

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.75
Professional:	.50
Other:	.25

Project Description:

Objectives:

To review the literature for evidence of offspring effects on parents; to provide a conceptual scheme to organize findings; to initiate studies in areas crucial to theories of early development.

Methods Employed:

Literature search, concept development, and development of research methods relevant to offspring effects on parents.

Major Findings:

The major effort this year was directed to laying the groundwork for a theory of how infants and young children affect their parents and caretakers in the first three years of life. There is not sufficient research at this time to develop an adequate theoretical model but it appears possible to gather together the conceptual elements needed, and place them in some rough order. The effects of the young can be seen in three ways: In launching the basic mother-infant relationship; in maintaining the relationship; and in terminating the relationship. Within each of these areas their effects operate in the context of "bouts" of interaction, to which the young again contribute by initiating, maintaining, and terminating bouts. To illustrate, in the case of launching the basic relationship, it is hypothesized that the pregnancy, the infant's physical appearance, the helpless thrashing movements, as well as the fact that the infant's sensory and motor system matches that of the mother, all contribute to launching caregiving, defined as the provision of life support and protection. As the behavior of infants becomes increasingly organized, early items in a sequence become discriminative stimuli for maternal avoidance behavior, so that much of caregiving consists of preventing undesirable and unpleasant outcomes (an aversive system). Caregiving is maintained, in part, because infants provide interpretable cues to their conditions, define their own sensory and fatigue limits, and maintain their protest behavior within the limits of the caregivers' tolerance. Their progressively discriminating attention to the caregiver, and their proximity-maintaining behaviors, tend to induce a singular one-to-one relationship with the caregiver, where this is possible.

The social interactions between the young and their parents, unlike caregiving, involve reciprocal exchange of behaviors with positive value, so that the contribution of the young lies not in providing signals to parents of aversive consequences that may develop. Infant behavior in this case generates appetitive parental behavior. It is felt that the young contribute to social interactions by first of all reducing the demand for caretaking (by being in states that favor mutual exchange); by being susceptible to manipulation of states; by being responsive in a very general sense; and by actively initiating social interactions.

Three general principles seem to emerge from a consideration of the various ways in which the young exert their effects in the aversive and appetitive systems. In both caregiving and social interactions the young change the general status of behavior in one period of development, so that their effects on parents in the subsequent period are different. Research strategies are needed that can detect these changing effects on the parent of emergent child behavior. Another very general principle is that the behavior of the young falls between the extremes of quantitative excess or deficiency, or inappropriate timing, relative to parent expectations. Extensions of this homeostatic model have proved helpful in explaining findings in the literature on socialization.

Finally, reflection on the nature of controls exerted on parent behavior by the young leads to the thought that there may be a balance of controls due to the fact that the greater maturity and long-term intentional behavior of parents is offset by the sheer volume of interaction started by the young, by the compelling nature of their behaviors, and by the way they selectively reinforce parental behavior.

The foregoing points have been developed in a series of colloquia presentations this year, and will appear as the lead chapter, now in draft stage, for a volume reporting on the results of a conference at Princeton devoted to effects of the young on caregivers.

Significance to Biomedical Research and the Program of the Institute:

This series of conceptual, methodological and empirical contributions is intended to bring about a revision of socialization theory and research methods that have, in the main, proved relatively unproductive in recent years. The revised approach may make it possible to launch socialization research on a course that corresponds better to the realities of parent-child interaction, and thus increase the likelihood of generating more consistent results than in the past. Socialization research is, in turn, the basis for advice to parents as well as the basis for many social and family-oriented action programs.

Proposed Course of the Project:

One remaining conceptual paper remains to be written. This paper will review various aspects of the importance accorded the young in historical documents from ancient Greece through to nineteenth century Europe and the United States. This task should be completed some time in the next year, after which the project will turn to empirical studies designed to test concepts that have now been developed.

Honors and Awards:

Richard Bell was elected to the governing council of the Society for Research in Child Development.

Publications:

Bell, R. Q. Hardy responses and fragile findings. In Bosma, J. F. (ed.): Third Symposium on Oral Sensation and Perception. Springfield, Ill., Charles C. Thomas, 1972, pp. 447-449.

Bell, R. Q. Detection of cross-stage relations between transition periods in which the form of behavior differs markedly: approaches used and findings from a longitudinal study of the newborn and preschool periods. In Monks, F. J., Hartup, W. H., and de Wit, J. (eds.): Determinants of Behavioral Development. New York, Academic Press, 1972, pp. 479-484.

Serial No. M-CR-23 (c)

1. Child Research Branch
2. Section on Parent-Infant Behavior
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Determinants and Dimensions of Mother-Infant Interaction

Previous Serial Number: Same

Principal Investigator: Howard A. Moss

Other Investigators: None

Cooperating Units: None

Man Years:

Total	3.00
Professional:	1.00
Other:	2.00

Project Description:

Objectives:

The major objectives of this research are to identify primary dimensions of mother-infant interaction, to investigate the determinants of these interaction patterns, and to relate these findings to data provided by other sections of the Branch longitudinal program.

Methods Employed:

The data collection for this project has been completed and we currently are engaged in data preparation and analysis. The sample consists of 121 mother-infant pairs (64 male and 57 female infants) from the second cohort of the Branch's longitudinal program.

All the procedures administered in the main longitudinal study of mother-infant interaction have been described in detail in previous annual reports. Briefly, these consisted of pregnancy interviews and questionnaires, 3-month home observations and laboratory procedures, and an 11-month home visit covering structured and free play behavior. Therefore this annual report will only provide a summary of our procedures.

In addition, we have completed data collection on a subsample of 36 second-born mother-infant pairs for whom we have antecedent data on the first-born. Two six-hour home observations of maternal-infant interaction were made using the same measures employed in studying the first-born sample. Following the second observation, the mother was interviewed briefly about differences and similarities between her experiences with her two infants. The main interest in studying this sample is to assess differences in maternal behavior toward first and second borns, using the mother as her own control. This second-born sample was selected so that we have sub-samples of second-born males with first-born brothers and first-born sisters, and second-born females, also, with first-born brothers and first-born sisters.

The home observations were coded for 41 variables involving either maternal or infant behaviors. In turn, these 41 variables have been combined in various groupings to produce an additional list of 56 combination variables, either through summarizing conceptually related individual measures (such as mother talks, imitates, and smiles at infant--to create a general measure of maternal social behavior); or through deriving new measures based on ratios in order to control for the base rate of an infant behavior which might be a factor influencing the maternal output of a particular behavior. An example of the latter type of measure is to express a mother's score for "imitates infant's vocalization" as a function of the frequency with which her infant vocalized.

The total number of 97 home observation variables is unwieldy, both from a statistical and from a theoretical viewpoint, and thus an important task confronting us is to reduce this list of variables, either through elimination of measures based on their relative utility, redundancy, or statistical shortcomings, and through summarizing procedures such as factor analysis.

The following list outlines the basic steps and types of analyses that will be conducted.

1. Assessment of measures for socioeconomic status (SES) differences.

2. Assessment of measures for sex differences.

3. Analysis of relations across measures in order to explicate concepts, and to determine construct validity.

4. Analysis of relations between maternal and infant behaviors to identify interaction patterns.

5. Short term predictive analysis to determine effects of maternal attitudes and behaviors on infant functioning.

6. Statistical treatment of measures directed toward data reduction, and production of summary variables.

Some of these analyses will be aimed at replicating findings obtained with previous samples studied at the Child Research Branch. Other analyses will be directed at providing further information on topics that interest us, such as the extent and effect of ambient and direct stimulation on the infant, precursors of social behavior or vocal behavior, the effect of contingent maternal behavior, infant protesting, etc. These analyses will be conducted separately for the sexes as well as by SES groupings in order to control for the variance associated with these demographic factors. The dimensions and concepts that are established through these within-stage analyses will be the basis for cross-stage analyses with other projects in the Branch's longitudinal program.

In a pilot study we observed that two marriage factors, identified as (1) Closeness to Husband's Family, and (2) Traditional Role Orientation were significantly associated, approximately two years later, with the mother's responsiveness to her infant's protest behavior at three months of age. Data collected on the main sample will be analyzed to determine if this result can be replicated.

Some preliminary results that we have obtained concern SES differences. We dichotomized our sample on SES and compared the absolute level, as well as pattern of interrelations, for scores on pregnancy interview ratings and home observation variables for these two SES groups. In the case of the pregnancy ratings, the upper SES mothers were rated significantly higher on variables dealing with interest in affectionate contact and social interaction with the infant, stimulation of the infant, and identification with the father. The lower SES women, on the other hand, were rated higher on variables measuring depression during pregnancy, apprehension over health and well-being of the infant, and degree of generalized apprehension. On a small sub-set of our home observation variables the upper SES subjects (females only) had significantly higher scores on variables assessing both Infant Smiles and Mother Smiles. Furthermore, correlations between the pregnancy ratings and maternal behavior in the home tended to be significantly related only for the upper SES mothers. Those correlations that did occur had rather evident face validity. For example the pregnancy rating on Interest in Affectionate Contact with Infant was significantly correlated with the frequency with which the high SES mothers "kissed" their infants during the 3-months home observations.

Preliminary data also is available comparing the sample of second-born cases we studied with their first-born sibs on the three-month home observation variables. These data show that mothers spend considerably more time caretaking and engage in more social-affectionate interactions with their first-born than their second-born offspring. On the other hand these mothers proffer more stimulation of other kinds to their second-borns.

Significance to Bio-Medical Research and the Program of the Institute:

The data collection from this project should provide basic information concerning the extent to which early response tendencies in infants, and various parental characteristics, conjointly contribute to early mother-infant interaction patterns. From these data we can begin to determine the variance in the mother-infant interaction that is attributable to infant characteristics, as well as the variance associated with particular maternal predispositions. The results of these studies should also help us understand the development of the mother's and infant's attachment toward one another; the emergence of social behavior and responsiveness in the child, and some of the developmental antecedents of cognitive functioning. The results should also provide information concerning the basic dynamics of early personality functioning.

Proposed Course of Project:

All data has been put on IBM cards except for some factual scores extracted from the pregnancy and postpartum interviews. These remaining data will be processed in the near future. Within-stage analyses have been initiated and some preliminary results have been produced. This coming year largely will be devoted to carrying out the various within-stage analyses listed in the methods section. During this period we will also begin some of the cross-stage analyses that we have planned. Examples of these analyses are studying the relation between marital factors and maternal responsiveness, and evaluating the degree and manner in which contingent maternal behavior, assessed during the infancy period, shapes pre-school socialization and verbal development.

Honors and Awards:

None

Publications:

Moss, H. A. Communication in Mother-Infant Interaction. Academic Press, In Press.

Serial No. M-CP(C)-18-3
1. Laboratory of Clinical Psychobiology
2. Section on Clinical Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical and Pharmacological Studies of Sleep

Previous Serial Number: Same

Principal Investigator: Frederick Snyder, M.D.

Other Investigators: William E. Bunney, Jr., M.D., J. Christian Gillin, M.D., Frederick K. Goodwin, M.D., Robert M. Post, M.D., Richard J. Wyatt, M.D., Robert I. Henkin, M.D., Robert Vigersky, M.D.

Cooperating Units: Section on Psychiatry, Laboratory of Clinical Science, NIMH
Laboratory of Clinical Psychopharmacology, SMHR
Experimental Therapeutics, National Heart and Lung Institute, NIH

Man Years (1972):

Total :	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

Using various biochemical and pharmacologic techniques in normal controls and patients with different disorders, we have previously found that both serotonergic and adrenergic systems appear to influence the occurrence of REM sleep. The relationship of delta (slow-wave) sleep to these systems and to diurnal changes in various hormone secretory rates is much less clear. Thus, the pursuit of the biochemical and physiological bases of sleep, abnormal and normal, continues as the fruitful implications of previous studies are followed up.

Patient Material:

4 medical patients
2 narcoleptics
5 volunteers
5 psychiatric patients

Methods and Findings:

A. Relationship of Serotonin to Narcolepsy

Two patients with narcolepsy have been intensively studied while receiving parachlorphenylalanine (PCPA) an inhibitor of tryptophan hydroxylase, or 5-hydroxytryptophan (5HTP), the precursor of serotonin. These results, together with those of one previously studied patient, are confusing. While receiving PCPA, two of the patients appeared to be less alert, more drowsy, and intellectually slowed down. Administration of 5-HTP to one patient did not reverse these effects; in fact, the mental changes were exacerbated. The third narcoleptic patient appeared to be improved by PCPA administration. When receiving 5-HTP alone, however, two patients became less alert and had difficulty concentrating. One patient briefly developed sensory illusions. A complete interpretation of these results awaits a full examination of our sleep data.

B. Effects of L-Histidine on Sleep

As reported previously, L-histidine, at a dose of 20gm/day, had few effects on the sleep of narcoleptic patients. During the past year, we administered L-histidine at a dose of 32gm/day to four normal volunteers, and 40gm/day to one medical patient. Again there was little evidence that L-histidine affected sleep. Additional subjects are being studied.

C. Effects of Thyrotrophic Releasing Factor (TRF) and Melatonin on Sleep

TRF and melatonin were administered intravenously on different nights to two psychiatric patients prior to sleep. No effects on sleep were noted.

Significance to Mental Health Research

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of central nervous system biochemistry and physiology to sleep and to mental illness. The studies of narcolepsy, in particular, bear directly on the understanding of these relationships.

Proposed Course of Project

The continuation of some of these and similar studies is planned during the next year as appropriate medical and psychiatric patients are referred. The collaboration of this Laboratory in such studies with the Laboratory of Clinical Psychopharmacology, SMR, will continue.

Publications:

Gillin, J. C., Post, R. M., Wyatt, R. J., Goodwin, F. K., Snyder, F., Bunney, W. E., Jr.: REM inhibitory effect of L-dopa infusion in humans. Clin. Electroenceph. Neurophys., in press.

Honors and Awards: None

Serial No. M-CP(C)-18-8
1. Laboratory of Clinical Psychobiology
2. Section on Clinical Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychophysiological Studies of Sleep and Waking in Normal Subjects

Previous Serial Number: Same

Principal Investigator(s): Frederick Snyder, M. D. and
Bernard L. Frankel, M. D.

Other Investigators: Evelyn L. Elwell (guest worker), Rona Buchbinder,
Andrew G. Morrow, M. D.

Cooperating Units: Surgery Branch, National Heart and Lung Institute, NIH

Man Years (1972):

Total:	2.0
Professional:	0.5
Other:	1.5

Project Description:

Objectives:

The two studies in this project share in common the purpose of seeking sleep-related correlates of stressful situations or processes. The first investigation, undertaken by Ms. Evelyn Elwell, studies the changes in sleep patterns and mental functioning of patients after open-heart surgery. The second study, being carried out by Ms. Rona Buchbinder, focuses on the changes in sleep patterns in relation to the phases of the normal menstrual cycle.

Patient Material:

6 open-heart surgery patients
10 normal subjects

Methods and Findings:

A. Polygraphic Study of Sleep in Selected Cardiotomy Patients

For four consecutive nights prior to open-heart surgery in six patients, nocturnal polygraphic sleep recording are being obtained. After surgery, the patients' sleep patterns are recorded continuously in the recovery room and again for four consecutive nights after leaving the recovery room. Behavioral observations and psychiatric evaluations are also made in the pre- and post-surgery periods. Preliminary analysis of the data from one patient reveals a decrease in REM sleep and an increase in the disturbance index after surgery. These findings may reflect the interruptions in sleep required for nursing and medical therapy. Data from five more patients are being collected and analyzed.

B. EEG - Sleep Patterns in the Phases of the Normal Menstrual Cycle

It is known that many women experience changes in psychological functioning during the various phases of their menstrual cycles. A few preliminary studies in other centers have reported menstrual cycle-related changes in some of the stages of sleep as recorded on the polygraph. It is possible that alterations in sleep patterns may partially underlie or be associated with some of these cyclical psychological changes. Normal healthy young women who have regular menstrual cycles and do not take oral contraceptives are sleeping at night in the laboratory during a complete cycle (or 35-40 consecutive nights) while polygraphic recordings of their sleep patterns are obtained. To help determine ovulation, subjects record their basal body temperature each morning. They also complete a daily questionnaire about the previous night's sleep. Preliminary data are not yet available.

Significance to Mental Health Research:

The study of the changes in sleep patterns in open-heart surgery patients is teasing apart the sleep-disruptive effects of routine post-operative medical and nursing procedures from those related more to the inherent stress of major surgery. If post-cardiotomy sleep deprivation and the associated disturbances in mental functioning are indeed demonstrated, then it may be possible to consider modifications in post-operative care so as to minimize sleep loss and its attendant psychophysiological morbidity. The delineation of the variations of the menstrual cycle-related patterns of sleep is expected to help elucidate the inter-relationships between sleep, hormones, and mental functioning.

Proposed Course of Project:

During the next year, Ms. Elwell's study will be completed and no further studies along these lines are planned. It is probable that this initial menstrual cycle study will also be completed. Further studies in this direction, however, may be undertaken if they are warranted by the results of the present one.

Publications: None

Awards and Honors: None

Serial No. M-CP(C)-18-69
1. Laboratory of Clinical Psychobiology
2. Section on Clinical Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Longitudinal Studies of Sleep and Concomitant Biological Changes in Psychiatric Patients

Previous Serial Number: Same

Principal Investigator(s): Frederick Snyder, M. D. and
Bernard L. Frankel, M. D.

Other Investigators: Kenneth Gaarder, M.D., Robert Coursey, Ph.D., Theodore P. Zahn, Ph.D., Carmi Schooler, Ph.D., Winfield H. Scott, Ph.D., Rona Buchbinder

Cooperating Units: Section on Clinical Psychology, CBRA
Laboratory of Psychology, CBRD, NIMH
Laboratory of Socio-Environmental Studies, CBRD, NIMH
Department of Psychology, University of Maryland

Man Years (1972):

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description:

Objectives:

The continuing purpose of this project is that of seeking objective correlates of impaired psychological functioning which will provide quantifiable assessments of diagnosis, clinical course, and effectiveness of therapeutic efforts over a wide range of disorders. While the recording of all-night sleep patterns from psychiatric in patients remains suspended as previously collected data is analyzed, the primary emphasis continues to focus on sleep and other psychophysiological studies of patients with chronic primary insomnia. These studies, undertaken by Dr. Bernard Frankel and several collaborators, are being carried out in conjunction with therapeutic trials of various non-pharmacological regimens, such as cerebral electrotherapy (also known as CET or electrosleep), electromyographic biofeedback, and autogenic therapy.

Patient Material:

24 insomniac patients
18 normal volunteers

Methods and Findings:

A study evaluating the effectiveness of cerebral electrotherapy in seventeen patients with chronic primary insomnia has been completed. The details of the protocol of this study were outlined in last year's annual report. In accord with preliminary findings, the final data analysis revealed that after treatment with CET, the insomnia patients did not show any clinically or statistically significant changes in polygraphic sleep record parameters (e.g., sleep latency, amount of total sleep, etc.), 24-hour urinary 17-hydroxycorticosteroid levels, self-ratings of anxiety and depression, and subjective assessments of sleep in comparison with pre-treatment measures of these same variables. Based on these data, it was concluded that cerebral electrotherapy is not an effective form of treatment for patients with chronic primary insomnia.

Another experimental therapeutic approach involves training insomniacs to learn to achieve states of deep muscular relaxation in order to promote better sleep. The training utilizes techniques of biofeedback and a type of self-hypnosis known as autogenic therapy. Twenty-four sleep-onset insomniacs (some of whom participated in the cerebral electrotherapy study) are being assigned to either a high- or low-anxiety group on the basis of Taylor-anxiety scale scores. Half of the insomniac patients in each group are to receive electromyographic biofeedback from the frontalis muscle. The other will take part in autogenic therapy. Both therapy protocols involve twice weekly sessions over a 6-week period and daily home practice sessions. Polygraphic sleep recordings as well as various psychophysiological data (including assessment of arousal levels by Drs. Zahn and Schooler) are being obtained before and after either course of therapy. In addition, the patients are keeping daily subjective sleep logs before, during, and after the period of relaxation training. Preliminiary results are not yet available.

A third study, near completion, compares various parameters of 18 patients with chronic primary insomnia and 18 age- and sex-matched normal "good sleepers." The results indicate that in comparison to normal subjects, the insomniac patients do indeed take longer to fall asleep, spend less of their total time in bed asleep, awaken more during the night, and have significantly less slow-wave (delta) sleep. These patients significantly overestimated sleep latency and underestimated both total time asleep and sleep efficiency. In contrast, the good sleepers underestimated sleep latency and overestimated total time asleep and sleep efficiency. In addition, the many significant differences between the scores of insomniac patients and good sleepers on various psychological tests tended to portray the "typical" patient with chronic primary insomnia as an anxious, depressed, hypochondriacal, uncomfortable, insecure, pessimistic worrier who is much more attuned to internal somatic stimuli and has a great deal of trouble relaxing. Twenty-four hour 17-hydroxycorticosteroid levels did not differ in the two groups. Analysis of data pertaining to average evoked response and rod-and-frame testing is not yet completed.

Significance to Mental Health Research:

The significance of the work of Dr. Frankel stems from the light it is beginning to shed on the nature of chronic primary insomnia, a common disorder about which there is relatively little scientific knowledge. In addition to the subjective complaints of the insomniac patient there is now objective documentation. It now appears that in addition to the marked decrease in total sleep, the significantly less delta sleep in these patients may be an important contributing factor in their feeling irritable, tense, depressed, tired, etc. The possible etiological significance of those personality factors which we assessed as deviant remains to be clarified. The demonstration that one new form of somatic therapy, cerebral electrotherapy, was not helpful in this disorder will hopefully encourage among other investigators recognition of the need for more carefully designed research studies of CET before definitive conclusions about clinical effectiveness (for any disorder) can be drawn. In this way, the public interest may be protected from several overzealous electronics companies who are marketing CET machines and basing unwarranted claims of efficacy and applicability on published studies suffering from serious methodological shortcomings which allow for ambiguous interpretations of the data. If any of the other experimental therapeutic approaches being studied should prove to be helpful for people with chronic primary insomnia then an alternative will have been found to the excessive use and occasional abuse of hypnotic and sedative medications in this pill-prone population of patients.

Proposed Course of Project:

Data analysis from the study of the comparison of sleep and other psychophysiological variable in insomniac patients and normal volunteer "good sleepers" will be completed, as will that from the extensive earlier studies of sleep in in patient psychotic patients. The evaluation of therapeutic effectiveness of biofeedback techniques and autogenic therapy as well as extensive pre- and post-treatment assessments of many psychophysiological parameters in patients with primary insomnia will continue during the next year. We also intend to undertake a more extensive study of the mechanism of action and possible therapeutic effects of various biofeedback techniques in patients under other stresses.

Publications:

Frankel, B. L., Buchbinder, R. and Snyder, F.: The effect of cerebral electrotherapy on the sleep of chronic insomniacs. In: Electrotherapeutic Sleep and Electroanesthesia. Proceedings of the Third International Symposium. Wageneder, F. M. and St. Schuy (eds.), Excerpta Medica, Amsterdam, in press.

Frankel, B. L., Buchbinder, R. and Snyder, F.: Treatment of chronic insomnia with electrosleep therapy. Sleep Research 1:168, 1972.

Honors and Awards:

Dr. Frankel will present a paper, "Sleep Patterns and Psychological Test Characteristics of Chronic Primary Insomniacs," at the annual meeting of the Association for the Psychophysiological Study of Sleep, San Diego, May, 1973.

Serial No. M-CP-18-6
1. Laboratory of Clinical Psychobiology
2. Section on Comparative Studies
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Comparative Studies of Sleep

Previous Serial Number: Same

Principal Investigator: Frederick Snyder, M. D.

Other Investigators: Nellie Bugbee, M.A., Jorge Perez-Cruet, M. D. and Thomas C. Douthitt, Ph.D.

Cooperating Units: Section on Technical Development, IRPD, NIMH
Laboratory of Clinical Science, CBRD, NIMH
National Zoological Park, Smithsonian Institution, Washington, D. C.
Division of Research Services, Veterinary Resources Branch, NIH, Poolesville, Maryland

Man Years (1972):

Total:	3.5
Professional:	1.5
Other:	2

Project Description:

Objectives:

Our studies of several small mammals have revealed features of their sleep habits which deserved further investigation. One such animal, the primitive rodent *Aplodontia rufa* or mountain beaver, has interested us because of his prominent ultradian cycling which is apparently independent of imposed light-dark cycles. We have attempted to discover if there is some external regulating influence to which these animals respond, and, if endogenously driven, the cycle can be affected by pharmacologically altering the metabolic rate. The hibernation tendencies of the European hedgehog have been studied both to discover what environmental changes induce hibernation in these animals and to relate hibernation as a behavioral state to the continuum of waking and sleep states normally shown by this animal. We have examined the sleep patterns of the monotreme *Tachyglossus aculeatus* or echidna, the only mammal yet studied who reportedly has no REM sleep. We have investigated this question using telemetry in order to minimize human interference and have allowed our specimen a long period of adaptation to the laboratory.

Studies of the effects of stress upon sleep patterns in rats have been carried out because initial evidence showed a significant reduction in REM sleep following periods of immobilization stress. Finally, we have undertaken studies of the behavioral and EEG effects of several of the prostaglandins in rats and cats. These cyclic fatty acids have recently been implicated as biochemical modulators of neurotransmission. Our studies have examined both acute and chronic effects on the EEG and upon patterns of sleep and waking.

Methods Employed:

All of the experiments on the sleep of animals have employed standard surgical techniques for the placement of chronic recording electrodes for EEG and EMG and thermistors for recording body temperature. In the animals recorded by telemetry the transmitting device is cemented to the skull bone and the battery is implanted subcutaneously in the fatty layers of the back. In the studies on hibernation, stress and prostaglandins standard cable recording was used. In all cases the animals were allowed to move freely within the confines of the recording chamber. Hibernation recordings were done in a temperature-controlled refrigeration room.

Major Findings:

A. Phylogenetic Studies

Our studies of the mountain beaver have demonstrated the existence of a striking ultradian alternation of sleep and waking which persists with only minimal changes even in the face of alteration of light-dark cycles, REM deprivation, and exposure to cold. The average period length of the cycle obtained from autocorrelational analysis of from 4 to 8 days recording from 11 specimens is 5.27 hours (range 4-6 hr.). The highest positive coefficients ranged from +.20 to +.45 indicating considerable variation in the daily regularity of the rhythm. Since this finding suggests the absence of a regularly recurring external driving force, we studied the degree of correspondence between the cycles shown by a number of animals being recorded simultaneously under the same experimental conditions for several consecutive days. Cross-correlational analysis for hourly percentages of waking were performed on 16 pairs of such recordings to discover the degree to which the animals cycled in phase with each other. Six of the coefficients were negative, 2 significantly so, and only one was significantly positive. The average coefficient was +.04 indicating that there is no correspondence between the cycles of the animals and thus it is highly improbable that they occur in response to an external stimulus.

Our investigation of endogenous influences has begun with studies of the effect of increasing metabolic rate by injecting thyroxin. When the effect of the treatment became evident as indicated by a 40% increase in heart rate, the cycling also became accelerated, increasing from baseline values of 4-5/day to 6-7/day. The amount of slow-wave sleep was not affected by the treatment, but REM sleep was clearly suppressed. Less than half the

the normal amount of REM sleep occurred. Percentages of waking were not altered, but twice as much time was spent in a transitional state corresponding to drowsiness.

The sleep of the monotreme, echidna (Tacyglossus aculeatus), has been studied because this very primitive and somewhat reptile-like mammal reportedly has no REM sleep although possessing all of the brain structures which have been shown to be involved in this function. Our specimen was allowed a long period of adaptation to life in captivity. It was provided with an enclosure having a deep layer of soil for burrowing and was recorded with telemetry to minimize restriction and human interference. Its diet was carefully controlled and it was apparently in good health during the period of our recordings. Even so, no evidence of REM sleep was obtained in a series of 24 hour recordings carried out over a three week period. Slow wave sleep was well developed and usually occurred in only one episode per day during the light phase.

A remarkable feature of this animal's behavior was the tremendous amount of time he spent awake (up to 82% per day). Our percentages are in aggreement with other reports so our specimen was apparently not abnormal in this respect. The echidna does not require a great deal of food, he is fossorial and very well-defended with sharp spines, so there is no ready explanation for the amount of vigilance he displays. A great deal of the time spent awake was engaged with digging and moving about in the recording box. Except for the complete absence of REM sleep, the sleep percentages obtained from this very primitive form are very much like those we have seen in a highly evolved animal, the goat. Again here is clear evidence that the evolution of sleep is far from a simple monotonic function of phylogenetic progression, but is rather a complicated product of specialized behavioral adaptations to diverse environmental challenges which have molded the behavior of each species throughout its history.

B. Relationship between Sleep and Hibernation

In our studies of the effect of ambient temperature on sleep, we have focused on a small hibernator, Erinaceus europaeus, the European hedgehog. Seven of these animals were housed individually in a refrigeration unit. All were implanted with electrodes for EEG, EMG, EKG and a thermistor for recording body temperature.

After several 24 hour baseline recordings had been collected of the hedgehog's sleep at a normal 72° F, the temperature was lowered at weekly intervals so that recordings were obtained at 60°, 50°, 40°, and 35° F. Of the animals thus far studied, two have clearly hibernated and both of these within 36 hours of the drop from 50° to 40° F. The remaining animals did not hibernate regardless of how long they were kept in the 40° condition or even when the temperature was lowered to 35° F.

As the room temperature was brought down, the normal body temperature and sleep patterns of the hedgehogs were disrupted. At 40° F, the animals slept only 80% as much as they did at 72° F. This was perhaps because increased physical activity was necessary to generate body heat, but in spite of this additional waking time, the body temperature of most of the hedgehogs declined significantly. One animal dropped from a normal value of 92° F to 83° F after several days in a 35° room.

The two animals which entered hibernation did not show a gradual decline in body temperature. They maintained normal levels until entering hibernation, at which point their temperatures plummeted to slightly above room temperature within the period of a few hours. Concurrently, the cortical EEG became isoelectric and the heart rate decreased from normal values of 220 bpm to 25 bpm. The state of hibernation was entered directly from a state of waking. The hibernating animals were apparently insensitive to external stimulation, loud noises and handling eliciting no arousal response, but nevertheless occasional brief spontaneous arousals were observed. Analysis of the heart rate in one subject indicated the perseverance of a circadian rhythm even during hibernation.

C. Effects of Immobilization Stress on Sleep in Rats

Earlier work from our laboratory has shown that rats subjected to repeated periods of immobilization show profound behavioral and biochemical changes. We were eager to learn how this stress affected the sleep of rats, since it is generally believed that, in humans, periods of stress are reflected by disruptions in normal sleep patterns.

Five rats were tied to a restraining board for two hours a day for 28 days. Their sleep was recorded for a baseline period, throughout the period of stress, and for a month after stress was terminated. The sleep of an unstressed control group was also monitered for the same period.

All the experimental rats showed an immediate effect of the stress in that their REM time was greatly reduced for the four hour period immediately following immobilization. For the first three weeks of stress all of the subjects were able to compensate for this deficit, so that for the entire 24 hour period following stress they had as much slow wave sleep and REM as on a normal, pre-stress day. But in the last week of immobilization, two rats no longer demonstrated this ability to compensate; although slow wave sleep remained stable, REM in these animals dropped to 50% of baseline values. Behaviorally, these two rats were notable in that they became extremely lethargic, lost weight, and ceased normal grooming activity. After the immobilization was ended, these rats continued to show behavioral deficits and drastic REM reduction.

These findings indicate that sleep is not a particularly fragile phenomenon. There could be little doubt that enforced immobilization was extremely unpleasant for our subjects, as evidenced by their continual struggles for release, intense vocalizations, and high rates of urination and defecation. Nevertheless, sleep was not chronically altered by this treatment. As for the two animals who did show REM reduction, this deficit did not appear until the end of a month of stress--a time when they were already exhibiting marked behavioral deterioration.

Thus, for lower mammals, it may be necessary to view changes in sleep not as an "early warning sign" of physiological stress, but as an indication that profound injury has already been done to the organism.

D. The Effects of Several Prostaglandins on the EEG of Rats and Cats

Prostaglandins are naturally occurring fatty acids which are found in one form or another of their 16 varieties in most structures of the body. Recent studies have indicated the important role they play in neurotransmission as a sort of biochemical modulator of the release of transmitter substances. Pharmacological investigations have shown rather dramatic behavioral effects after administration of these compounds, especially PGE_1. In collaboration with Dr. Perez-Cruet, we have studied the effects of four of the prostaglandins on the EEG of rats and cats and the acute effects of their administration on subsequent sleep and waking patterns. The compounds studied were PGE_1, PGE_2, PGF1a, and PGF_{2a}. With only minor variations and exceptions which will be pointed out, the effects to be described were the same in both rats and cats, and the differences among the several compounds were mainly ones of intensity and duration.

All four prostaglandins produced an immediate EEG desynchronization accompanied by a marked reduction of EMG amplitude. The behavioral response resembled sedation. The animals were unresponsive, showed extreme relaxation, and at the peak of the effect were prostrated. These prostaglandins also produced transient autonomic signs such as plantar erythema in the rats and occasionally diarrhea. Vomiting occurred in some of the cats, especially after the PGE compounds which seem to be more potent.

The behavioral and EEG effects, which long outlasted the somewhat explosive autonomic response, resembled the state of paradoxical sleep in the cats wherein cortical desynchronization and muscular relaxation are quite marked. However in rats a hypersynchronous theta pattern driven by the hippocampus is the EEG sign of paradoxical sleep, and this pattern did not occur in response to the prostaglandins in these animals. While apparently sedated, their EEGs were completely desynchronized and except for being somewhat flatter and more invariant (frequencies in the beta range were predominant) were indistinguishable from waking. This effect lasted about two hours after PGE_1 and for little more than 30 minutes with PGF_{1a}. Subsequent to this drug effect, normal alternation of sleep and waking states with normal expression of paradoxical sleep was observed. The drug treatments had apparently no more effect upon this later behavior than did injections of the vehicle alone.

We have attempted to further define the mechanism of action of the prostaglandins by administering other drugs with known effects upon central nervous system activity in combination with PGE. Cholinergic blockade with atropine sulfate blocked or suppressed the EEG desynchrony observed in the cats without affecting the muscular relaxation. The effects of atropine were less pronounced in the rats. Blockade of catecholamine synthesis with alpha-methyl-para-tyrosine did not affect the response, but administration of harmaline, a reversible monoamine oxidase inhibitor, completely suppressed the behavioral sedation to the extent that the animals became hyperexcitable. We are currently investigating the chronic effects of blocking agents specific to the prostaglandins such as indocin and the centrally-acting acetaminophen. Neither of these agents has been effective in suppressing REM sleep. At this point we are led to conclude that the resemblance between the prostaglandin response and paradoxical sleep, while probably sharing some underlying neurochemical mechanisms, is no more than superficial.

Significance to Mental Health Research:

The variety of studies being carried out with animal subjects in this laboratory serve to provide information about evolutionary trends in sleep habits and about the extent to which these habits can be modified by controlled environmental changes or by pharmacological intervention. While substantial evidence accumulates defining the various roles of different biochemical substances in the mediation of the several states of sleep, again and again species differences appear which must be important for an understanding of sleep as a biological phenomenon. There are numerous reasons, both theoretical and methodological, which require that comparative studies of a variety of species be carried out.

Proposed Course of the Project:

While continuing to pursue the descriptive aspect of the phylogenetic studies carried out both in the field and in the laboratory, a study of responsiveness to stimulation in sleep using a conditioned avoidance response to evaluate level of performance after awakening is now in its preliminary stages. Although the procedure will first be used with rats, we trust it will be suitable for use with many other species whose sleep we have studied in other respects.

Publications: None

Honors and Awards:

A report describing the effects of the prostaglandins has been accepted for presentation at the annual meeting of the Association for the Psychophysiological Study of Sleep in San Diego, California in May 1973.

Serial No. M-CP-18-7
1. Laboratory of Clinical Psychobiology
2. Section on Comparative Studies
3. Bethesda, Maryland

PHS-HSMA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Predatory Aggression in the Rat

Previous Serial No.: Same

Principal Investigator: Thomas C. Douthitt, Ph.D.

Other Investigators: Jorge Perez-Cruet, M. D.

Cooperating Units: Laboratory of Clinical Science, CBRD, NIMH

Man Years (1972):

Total:	1
Professional:	1
Other:	none

Project Description:

Objectives:

Previous work on aggression carried out in this laboratory by Dr. Burr Eichelman and colleagues and findings resulting from the research of several other laboratories have shown that an antagonistic interaction between serotonergic and cholinergic neurotransmission systems underlies the sort of predatory aggression expressed as mouse killing by rats. Serotonin is seen as an inhibitor, while acetylcholine acts to facilitate this behavior. The evidence for the actions of these substances is basically pharmacological since mouse killing is either produced or blocked by the administration of drugs which act upon these two neurochemicals either to enhance or to reduce their normal level of activity.

Our current efforts have centered not only upon an extension of the pharmacological approach to understanding the mechanisms responsible for mouse killing; i.e., by producing it with drugs, but also to study various factors influencing this behavior in animals who kill spontaneously. Possible methods of blocking the behavior in both classes of mouse killers are employed to determine the extent to which pharmacologically induced and "spontaneous" killing are comparable and responsive to treatment by use of drugs or by modification of factors which influence the behavior.

Methods Employed:

The testing procedure is simple. A mouse is presented to a rat in his home cage. The duration of the exposure varies according to the demands of the particular experiment, but latency to kill has proved to be a valuable measure so each test is timed. To date, only one strain of rats has been used: The Osborn-Mandel strain available from the NIH colony. This strain typically has a high proportion of mouse killers. This is a reliable and consistent feature of their behavior and conversely, if a subject does not kill upon being exposed to a mouse, there is little chance that he will ever do so. Each group of rats obtained can thus, after a few testing sessions, be separated into subgroups of killers and non-killers.

Since pharmacological evidence has implicated the involvement of several neurotransmitters in mouse killing, drugs are used which intervene in the normal functioning of these substances either by enhancing their potency by interfering with metabolic breakdown or stimulating their receptors, or by decreasing their influence by blockade of synthesis or receptors or, as in the case of two especially potent and useful drugs, by producing selective degeneration of either adrenergic or serotonergic nerve terminals in the brain.

Investigations of diurnal influences on the rate and rapidity of mouse killing in natural killers have been carried out as well as studies on the effects of several different light-dark schedules. Availability of food has also been a variable of interest.

Following completion of each experiment, biochemical determinations of brain levels and occasionally of synthesis rates of norepinephrine, dopamine and serotonin are performed both to confirm drug effects upon treated animals and to discover possible biochemical differences between the animals who kill and those who do not.

Major Findings:

A. Drug Studies

A new drug which produces possibly irreversible destruction of serotonergic nerve terminals, 5-6 dihydroxytryptamine (5-6 OH,HT), when injected intracerebrally to non-killer rats produces consistent mouse killing in a significant number of them (47%). We have also found that 6-hydroxydopamine (6-OHDA), which produces similar damage in adrenergic nerves and has only a negligible effect upon mouse killing by itself, powerfully enhances the effect produced by 5-6 OH, HT when injected in combination with that compound (71%). Levels of serotonin are lowered by 6-OHDA and dopamine is similarly affected by 5-6 OH, HT, but the effects observed after the combined drug treatment do not seem explicable on the grounds of simple additive effects. We have, for example, on the basis of evidence showing cholinergic mediation of mouse killing, administered atropine sulfate to killers produced so by either 5-6 OH, HT, 6 OHDA, or by the combination of the two. Atropine is effective as a blocking agent only in the latter two groups, those wherein there is considerable adrenergic destruction. Peripheral side-effects of this cholinergic blockade were suspected but ruled out when atropine methyl nitrate, which does not cross the blood brain barrier, completely failed to block the killing exhibited by these animals.

Parachlorophenylalanine (PCPA), which inhibits the action of the enzyme tryptophan hydroxylase and thus blocks the synthesis of serotonin, produces mouse killing in rats as impressively as does 5-6 OH, HT and similarly, the effect is not reversed by atropine. In contrast to our findings with the 5-6 OH, HT -- 6-OHDA combination, however, the administration of an agent which blocks the synthesis of catecholamines, alpha-methyl-paratyrosine, blocks instead of enhances killing in animals treated with PCPA. This finding suggests that although ultimate depletion of catecholamines is accomplished by both blockade of synthesis and by nerve terminal destruction, markedly opposite effects upon predatory aggression are obtained and thus reduction in the levels of available neurotransmitters is not the crucial factor.

The possibility exists that a functional super-sensitivity develops in receptive structures following biochemical denervation with 6-OHDA. Since our findings have led us to conclude that in animals where serotonergic and cholinergic mechanisms have been disrupted, the adrenergic system is fully capable of driving mouse killing behavior, it is hypothesized that it is receptor super-sensitivity to remaining levels of norepinephrine which supports and can even enhance the mouse killing produced by depletion of serotonin. Preliminary results with a group of animals treated with 5-6 OH, HT have shown that mouse killing can be significantly reduced by alpha adrenergic receptor blockade with dibenzyline. Beta adrenergic blockade with propranolol is not effective. The involvement of adrenergic receptors in the maintenance of mouse killing is thus fairly certain and studies are in progress to further elucidate their function in this respect.

B. Environmental Influences

It is well known that diurnal cycles are a prominent feature of practically every biological phenomenon. Mouse killing by rats is no exception. We have demonstrated this by using a group of rats who kill mice spontaneously, matched with a group who do not normally kill. The animals were tested at six hour intervals under an artificial twelve hour light-dark schedule which was reversed for one testing, and under conditions of constant light and constant darkness. Aggression, as measured by both frequency and latency of mouse killing was most facilitated by darkness no matter what the actual time of day. Shifting the hours of darkness also shifts the peak incidence of killing, and the highest frequencies and lowest latencies were obtained under conditions of constant darkness. After several days of adaptation in the dark even non-killer rats became more irritable and aggressive and the only kill ever observed in this group was obtained. Under conditions of constant light, it appears that natural clock time governs the effect since peak killing occurred during the nighttime hours. We are currently attempting to relate these effects to the diurnal variations occurring in the several biochemical substances which have been shown to be involved with mouse killing. Since rodents as a class generally behave nocturnally, it is not surprising that their aggressive behavior should also be so, but we were impressed by the extent to which this behavior could be manipulated by modifications of the cycles of light and dark.

Following the completion of these studies determination of fore- and hindbrain monoamine levels and turnover were carried out. While there were no differences in the basal levels, synthesis rates for both dopamine and norepinephrine were significantly elevated in the killer rats. Other findings indicate that on a feeding regimen of two hours food availability per day, mouse killing does not increase as a function of time since feeding, but we have not investigated the effects of long term food deprivation. Since the animals who consistently kill, do so when food is continuously available, and animals who do not are not driven to do so even after a 22 hour fast, we conclude that, within the limits of our testing situation, hunger is not an important drive supporting this behavior.

Significance to Mental Health

Mouse killing by rats is admittedly a far cry from the aggressive behavior of man which has both shaped and devastated his civilization. It does however provide a laboratory model of a behavior which is demonstrably under biochemical control, the understanding of which can only be valuable for controlling destructive aggressive behavior in man. The correspondence need not only be one between overt forms of aggression, however, since ability to suppress mouse-killing in rats has become an important pre-clinical test of the potential efficacy of anti-depressant drugs.

Proposed Course of the Project

Controlling the aggressive behavior of natural mouse killers is a more complicated matter than it is in animals who kill under the influence of drugs whose action is known. We believe that efforts must be made to describe the difference in the neurochemistry of killer and non-killer rats and are currently undertaking a study of acetylcholine levels in the brain and an analysis of brain and plasma amino-acid levels. Beyond the hope of discovering biochemical factors which distinguish these animals who differ so in this item of aggressive behavior, it has become increasingly clear that the problem of pharmacologically induced aggression is far from being solved. We intend to continue the studies relating serotonin depletion to aggression on the theory that it is the balance relating the remaining neurotransmitters and not the exclusive action of only one of them which determines the extent to which this form of aggression will become manifest.

Publications: None

Honors and Awards:

A paper describing the results obtained with 5-6 OH, HT and 6-OHDA entitled, "Evidence for an Interaction Between Serotonin, Catecholamines and Acetylcholine in Mouse Killing Behavior," was presented by Dr. Perez-Cruet at the Federation of American Societies for Experimental Biology 57th annual meeting in Atlantic City, New Jersey. (*Fed. Proc.*, 32: 368 abs., 1973)

Serial No. M-CP-18-8
1. Laboratory of Clinical Psychobiology
2. Section on Comparative Studies
3. Bethesda, Maryland

PHS-HSMA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pharmacological Studies of Responsivity and Arousal Level in Adult and Neonatal Rats

Previous Serial Number: None

Principal Investigator: Thomas C. Douthitt, Ph.D.

Other Investigators: Yvonne Brackbill, Ph.D. and Jorge Perez-Cruet, M.D.

Cooperating Units: Department of Obstetrics and Gynecology, Georgetown University,School of Medicine and Laboratory of Clinical Science, CBRD, NIMH

Man Years (1972):

Total	.8
Professional:	.6
Other:	.2

Project Description:

Objectives: The studies carried out this year have sought to discover, first, the role played by both central and peripheral adrenergic and serotonergic mechanisms in the control of the organism's response to novel stimulation and, second, how the neonatal maturation of central adrenergic neurotransmission systems is related to the environmental modulation of arousal level during early development. In the first instance, we are concerned with the biochemical basis of the orienting response (OR) of the adult animal by examining the roles played by the catecholamines and serotonin in the control of the cardiac deceleration which is a reliable indicator of the OR to novel non-aversive stimulation.

The study of central adrenergic mechanisms mediating stimulation effects on neonatal arousal level grew out of previous work carried out by Dr. Yvonne Brackbill on the psychophysiological effects of continuous stimulation in the human newborn. In general, it was found that stimulation produced a lowering of arousal level even to the point of inducing sleep. Preliminary longitudinal studies carried out on rat pups indicated that this behavioral suppression persisted only during the first 10 days of life following which point the same stimulation began to have an excitatory effect. Since this shift occurred at about the time when catecholamine levels in the brain begin to approach adult values, the present study was undertaken to determine if, in fact, central adrenergic mechanisms are responsible for this dramatic change in behavioral response.

Methods Employed: In the study of the adult cardiac OR, heart rate is measured in rats exposed to a 10 second, 1000 Hz tone at 70 db. In addition to a control group, there are four drug-treated groups. Central and peripheral adrenergic influences on the response are evaluated in two groups: one injected intracerebrally and the other intravenously with 6 hydroxydopamine (6-OHDA) which produces destruction of adrenergic nerve terminals and consequent lowering of catecholamine levels. Two other experimental groups were similarly treated with a new compound, 5-6 dihydroxytryptamine (5-6 OH, HT), which destroys serotonergic terminals. Neither compound crosses the blood-brain barrier. In all groups, heart rate during and after stimulation was compared to pre-stimulation level. Following the testing, brain and heart catecholamine levels were determined as well as brain and lung serotonin content.

A similar pharmacological approach is being used in the study of the effects of continuous stimulation on arousal level during early development in the rat. Here 6-OHDA is injected intracisternally to rat pups at 24 hours after birth. Litter mate controls, both vehicle injected and untreated, are used for comparison. A cross-sectional design is used so that testing is performed on a different set of treated and control animals on each day from ages 4 through 21 days. The indicators of arousal level are heart rate and electronic recording of gross motor activity. Testing sessions consist of exposure of the pups to 10 minutes of continuous white noise at 80 db and 10 minutes of no stimulation in a counterbalanced design. In order to determine the extent of the drug effect, brain levels of norepinephrine and dopamine are determined.

Major Findings:

A. Cardiac Orienting Response: During the 10 second presentation of novel auditory stimulation, the control animals showed a significant drop in heart rate beginning at about 5 beats after onset and continuing with some increase in variability throughout the duration of the stimulus. There was a further deceleration of comparable latency to the offset of the tone with a gradual return to baseline levels after 10-15 seconds. With the exception of the animals injected peripherally with 6-OHDA who showed no cardiac response to stimulation, this decelerative pattern of responding was predominant in all of the drug-treated groups, and no significant differences from the control response pattern were obtained. There were no significant differences among the groups either in baseline level or in the variability of the heart rate. Biochemical determinations of dopamine, norepinephrine, and serotonin in target structures confirmed that the drug treatments were effective since significant depletions were obtained.

We are led to conclude that since the peripheral treatment with 6-OHDA, which accomplishes a sort of chemical sympathectomy, is effective in blocking the cardiac OR, the bradycardia observed during orienting in the rat is sympathetically mediated. A diminution of sympathetic activity rather than an increase in vagal influence must be responsible for the response in the rat, an animal who is known to be sympathotonic. Since the OR is considered to be a centrally mediated response, it was surprising that neither of the centrally treated groups showed any change. It appears that neither a fully functional serotonergic or adrenergic system in the brain is alone responsible for the expression of the response. The possibility exists that central cholinergic blockade might affect it as well as might drug treatments which knock down several central neurotransmission systems in combination.

B. Continuous Stimulation and Neonatal Arousal Level: With the study nearing completion, the findings to date indicate that normal maturation of central adrenergic function is necessary for the occurrence of the shift from behavioral suppression to excitation in response to continuous stimulation. Although excessive variability has made heart rate an unreliable measure in this context, the effect is reflected in stabilimetric recording of gross motor activity. On days 4 through 10 animals treated with 6-OHDA respond in the same manner as do control animals: there is a suppression of activity during stimulation. Following 10 days of age, the control animals begin to become more active during stimulation, whereas those treated with 6-OHDA do not.

Significance to Mental Health Research:

Both of these studies have attempted to define the roles of important neurochemicals in the modulation of the organism's response to environmental input. Orienting to novel stimulation is a necessary antecedant to appropriate behavioral adjustment and the study of the mechanisms supporting it is basic to the understanding of normal and abnormal behavioral adaptations. The study of the ontogeny of response patterns to stimulation and their biochemical mediation is perhaps even more relevant the issue of normal adaptation. During a period in life when novel experience is the rule, it is of interest to know what sort of adjustments are made by the organism to stimulation and how they are mediated. By the use of 6-OHDA in these experiments, we have shown the importance of intact adrenergic functioning both for the cardiac OR and for normal motor responsiveness to simulation in early development.

Proposed Course of the Project:

The study of the OR in rats showed no effect of central adrenergic or serotonergic destruction. Since the OR is apparently intact in these preparations, but is normally quite a transitory response, it is of interest to study the pattern of its habituation in treated animals. The habituation of the OR is a primitive type of learning supposed by many to be necessary for the development of conditioning. Studies evaluating the influences of catecholamines and serotonin upon this phenomenon are being considered. The involvement of this laboratory in the neonatal study is nearing completion.

Publications: None

Honors and Awards:

A publication of two of the investigators, Drs. Yvonne Brackbill and Thomas C. Douthitt on neonatal arousal level (Brackbill, Y., et al.: Psychophysiologic effects in the neonate of prone versus supine placement, *J. Pediat.*, 82: 82-84, 1973) has been selected for inclusion in the *Yearbook of Pediatrics* for 1973.

Serial No. M-CS-OC(C)-04
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project title: Studies of the interrelationships of the nervous and circulatory systems.

Previous Serial Number: Same

Principal Investigator: Philippe V. Cardon

Other Investigators: James L. Weiss, Raymond J. Matta, Thomas N. Chase, G. Frederick Wooten, Friedhelm Lamprecht, Griff T. Ross, August Watanabe, Jorge Perez-Cruet.

Cooperating Units: Sections on Pharmacology, Experimental Therapeutics, Psychiatry, and Medicine, LCS; Reproduction Research Branch, NICHHD: Department of Medicine, University of Indiana.

Man Years:		
	Total	0
	Professional	0
	Other	0

Project Description:

Objectives: To study the interaction of the nervous and circulatory systems and the effects of drugs on those systems.

Methods Employed: Standard non-traumatic techniques for assessing well-known circulatory parameters in man. Direct measurement of analogous parameters in animals. Electrical recording of peripheral sympathetic nerve activity in animals. Enzymatic assay of blood. Radio-immune assay of pituitary trophic hormones in blood. Standard tests of renal function.

Major Findings: This project has been inactive this year because of Dr. Cardon's work on the research task force. Work will be resumed in the coming year.

Proposed Course of Project: Further studies of similar nature.

Honors and Awards: None

Publications:

James L. Weiss, M.D.; August M. Watanabe, M.D.; Louis Lemberger, M.D., Ph.D.; Norman R. Tamarkin, M.D. and Philippe V. Cardon, M.D.: Cardiovascular effects of delta-9-tetrahydrocannabinol in man. Clin. Pharmacol and Ther. 13: 671-684, 1972.

G. Frederick Wooten, M.D. and Philippe V. Cardon, M.D.: Plasma dopamine-β-hydroxylase activity. Arch. of Neurol. 28: 103-106, 1973.

Serial No. M-CS-OC(C)-16
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of pinched-off nerve endings (synaptosomes) as a model system for investigating the transport, binding and metabolism of biogenic amines in the central nervous system

Previous Serial Number: Same

Principal Investigator: Robert W. Colburn

Other Investigators: L.K.Y. Ng (Guest Worker), Irwin J. Kopin, Nguyen B. Thoa (Guest Worker), Jonathan Moss (Guest Worker), Gertrude D. Maengwyn-Davies (Guest Worker), Friedhelm Lamprecht, K.S. Rajan

Cooperating Units: Section on Experimental Therapeutics, LCS and Illinois Institute of Technology Research (Chicago)

Man Years:		
	Total	3.50
	Professional	3.25
	Other	0.25

Project Description:

Objectives: Synaptosomes isolated from whole brain represent an in vitro system similar in many respects to that present in living organisms. Current basic problems being investigated with this system are: (1) How do neurotransmitters pass through membranes? (2) How are they bound within vesicles? (3) What is the mechanism for their release? A concurrent study involves investigation of the effects of psychotomimetic drugs on the above parameters for neurotransmitter substances.

Methods Employed: Drugs used for treatment of depression or mania such as desmethylimipramine, lithium or narcotics are added to the synaptosome system. The uptake, binding and release of a variety of neurotransmitter substances are measured; alternatively, drugs are given in vivo prior to isolation of the synaptosomes. Synaptosomes are fractionated into plasma membranes, synaptic vesicles and solubilized receptor proteins. The chelation chemistry of neurotransmitters as a possible mechanism for binding and release in synaptosomes is studied.

Major Findings: The synaptosomes take up, bind and release monoamines such as norepinephrine and serotonin much as they function in in vivo or in perfusion experiments. As in the peripheral sympathetic nervous system, the route of norepinephrine metabolism varies with the mode of its release. Uptake studies with neurotransmitters are of importance since reuptake is a major means for termination. Recent findings with the in vitro synaptosome system provide evidence that the addition of certain toxins simulate electrical stimulation even in those isolated preparations. The subcellular localization of drugs such as marihuana, morphine and diphenylhydantoin and their effects on the binding and release of neurotransmitters have been determined. At the chemical level, current studies involve the coordination of neurotransmitters to metal ions and metal-ATP and phospholipid complexes.

Significance to Biomedical Research and the Program of the Institute: The drugs altering monoamine function have proved very useful in modifying mood. The present studies provide information on a molecular basis concerning drug action.

Proposed Course of Project: With the above system, large numbers of very similar samples can be prepared and will be used in comparing a wide variety of drugs using kinetic methods to determine active mechanisms. Synaptosomes will be further fractionated to isolate carrier or receptor proteins.

Honors and Awards: None

Publications:

Rajan, K.S., Davis, J.M., Colburn, R.W. and Jarke, F.H.: Metal chelates in the storage and transport of neurotransmitters: Formation of mixed ligand chelates of Mg^{2+}-ATP with biogenic amines. J. Neurochem. 19: 1099-1116, 1972.

Milne, G.W.A., Fales, H.M. and Colburn, R.W.: Chemical ionization mass spectrometry of complex molecules. Analyt. Chem., in press.

Serial No. M-CS-OC(C)-19
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Metabolism, distribution and biochemical effects of psychoactive drugs

Previous Serial Number: Same

Principal Investigator: Irwin J. Kopin and Julius Axelrod

Other Investigators: L.K.Y. Ng (Guest Worker), Robert W. Colburn, David S. Kreuz, Friedhelm Lamprecht, Redford B. Williams, Michael H. Ebert

Cooperating Units: Sections on Pharmacology, Experimental Therapeutics and Medicine, LCS

Man Years:		
	Total	1.5
	Professional	1.1
	Other	0.4

Project Description:

Methods Employed: (1) Drugs are administered; and using radioactivity or chemical methods, the levels in blood, urine and feces are determined. (2) The alterations in amine metabolism are assessed by examining the tissues and biological fluids of animals or the blood, cerebrospinal fluid and urine of man. (3) The modification of effects of one drug by another is examined in animals. (4) Mass spectroscopic and radioimmunoassay techniques for drugs are being developed.

Major Findings: Persistence of tetrahydrocannabinol in the blood may be related to its avid storage in fatty tissues of the body. In the brain, the drug is localized in synaptosomal fragments where it alters *in vitro* the rate of uptake and efflux of amine neurotransmitters. Animals treated with delta-9-tetrahydrocannabinol are more responsive to immobilization stress as indicated by increases in tyrosine hydroxylase in the adrenal medulla. Tetrahydrocannabinol causes hypotension in rats and prevents the elevation of blood pressure seen with repeated immobilization.

Significance to Biomedical Research and the Program of the Institute: The problem of drug usage is currently a major concern. The studies will help to elucidate the mechanisms of action of drugs which are abused, to indicate their possibly toxic effects and to suggest means for treatment of patients suffering from toxic effects of the drugs.

Proposed Course of Project: Identification of metabolites, estimation of drug effects on amine metabolism and enzymes concerned with amine synthesis and with degradation and examination of drug interactions will continue.

Honors and Awards: None

Publications:

Lamprecht, F., Kvetnansky, R., Ng, L.K.Y., Williams, R.B. and Kopin, I.J.: Effect of delta-9-tetrahydrocannabinol on immobilization-induced changes in rat adrenal medullary enzymes. Eur. J. Pharmacol. 21: 249-251, 1973.

Williams, R.B., Ng, L.K.Y., Lamprecht, F., Roth, K. and Kopin, I.J.: Delta-9-tetrahydrocannabinol: A hypotensive effect in rats. Psychopharmacologia 28: 269-274, 1973.

Serial No. M-CS-OC(C)-20
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Histochemical studies of biogenic amines

Previous Serial Number: Same

Principal Investigator: David M. Jacobowitz

Other Investigators: J. Steven Richardson (Guest Worker), Miklos Palkovits, Hiroshi Y. Watanabe, Paul D. MacLean, Lloyd A. Greene, Kenneth Kent, Theodore Cooper, Marshall W. Nirenberg

Cooperating Units: Laboratory of Brain Evolution and Behavior, NIMH; Laboratory of Biochemical Genetics, NHLI; Cardiology Branch, NHLI

Man Years:		
	Total	4.0
	Professional	3.0
	Other	1.0

Project Description:

Objectives: (1) A multidisciplinary approach to the study of the adrenergic nervous system is utilized with major emphasis on monoaminergic nerves in the brain. Localization of monoaminergic nerves (noradrenergic, dopaminergic, serotonergic) are accomplished first by histochemical means. Drugs which have the ability to selectively destroy noradrenergic or serotonergic nerves are administered to rats. Correlations of chemical and histochemical analyses of brain amines, monoaminergic neurons and behavioral observations are made. (2) Localization of cholinergic neurons in the brain by a histochemical method. This will provide the basis for the long-term objective for studying central adrenergic-cholinergic interactions. (3) Use of tissue culture to study nerve growth in organ and dissociated cell cultures. (4) On a long-term basis, this project represents an investigation of both the morphological basis for catecholaminergic mechanisms and the functional organization of the autonomic nervous system in several organs.

Methods Employed: (1) The morphological procedure involves the use of a specific fluorescence histochemical method for the demonstration of catecholamines (norepinephrine, epinephrine, dopamine) and indoleamines (serotonin) within monoaminergic nerves and cells. (2) Biochemical assays for norepinephrine, dopamine and serotonin are available. (3) The histochemical procedure for cholinergic neurons by the use of the acetylcholinesterase-staining technique. (4) Brain stereotaxic method for intraventricular injections of drugs. (5) Behavioral parameters such as water and food consumption, spontaneous motor activity, rectal temperature and emotional arousal are measured.

Major Findings: (1) 6-Hydroxydopa is a neurotoxic agent which selectively destroys a portion of the noradrenergic terminals in the central and peripheral nervous system. Injection of 6-hydroxydopa into the lateral cerebral ventricles of rats produces a graded dose-dependent reduction of norepinephrine in the telencephalon diencephalon, hindbrain and cerebellum. Dopamine levels are not altered by the doses of 6-hydroxydopa used. After an initial reduction, food and water consumption and spontaneous motor activity return to normal at a time when norepinephrine levels are still greatly reduced. Emotionality is increased by the injection of 6-hydroxydopa and returns to normal only after 70 days when norepinephrine levels in the telencephalon and hindbrain have also returned to normal. Norepinephrine concentration in the diencephalon and cerebellum remains significantly below control values 70 days after injection. It is suggested that norepinephrine activity in the telencephalon and/or hindbrain is an inhibitory component of emotional arousal.

Studies with 6-hydroxydopa in shock-induced aggression in rats suggest that this type of aggression is modulated through a central noradrenergic system.

Fluorescent histochemical observations indicate a marked reduction of noradrenergic terminals in certain brain areas and a buildup of catecholamines within axons, which suggests that 6-hydroxydopa has caused a degeneration of noradrenergic nerve terminals. The budding and sprouting of new varicose processes indicate that some of the nerve terminals destroyed by 6-hydroxydopa are replaced by the regrowth of catecholamine terminals.

5,6-Dihydroxytryptamine is a neurotoxic drug which is capable of destroying serotonergic nerve terminals in the brain. In rats treated with both 6-hydroxydopa and 5,6-dihydroxytryptamine, reduction of serotonin tends to restore the balance between the norepinephrine and serotonin systems and thus reduces the emotionality rating score. Consequently, the increased emotionality seen after 6-hydroxydopa might be due to the imbalance between brain noradrenergic and serotonergic systems.

(2) Because of the buildup of fluorescent catecholamines in noradrenergic nonterminal axons after 6-hydroxydopa injection, the norepinephrine-containing neurons of the rat brain were capable of being mapped. Rat brains were studied after an intraventricular injection of 6-hydroxydopa. The dorsal noradrenergic bundle was revealed and followed to its point of union with the ventral and dopaminergic bundle. The fasciculus longitudinalis dorsalis (Schutz) contains a noradrenergic tract that was followed to the hypothalamus. The cortex receives innervation from several routes: (a) via the median forebrain bundle (MFB) by way of the tractus diagonalis and the tractus septocorticalis around the genu of the corpus callosum through the cingulum, (b) via the MFB through the internal capsule and (c) via the dorsal bundle through the internal capsule. The hippocampus receives innervation via the MFB by way of the tractus diagonalis and fornix superior. The amygdala is innervated via the MFB and the stria terminalis (pars precommissuralis) through the nucleus interstitialis striae terminalis and caudally through the stria terminalis. The septal area is innervated via the MFB through the tractus diagonalis and tractus septohypothalamicus. The nucleus accumbens appears to receive processes via the

tractus striohypothalamicus. It is suggested that the thalamus may receive innervation via the MFB through the stria medullaris thalami and also via the dorsal bundle by way of the fasciculus retroflexus through the stria medullaris.

These studies demonstrate that 6-hydroxydopa is a valuable agent for the study of the influence of noradrenergic nerves on various modes of behavior in addition to its usefulness in mapping the noradrenergic axonal pathways of the brain.

(3) Regions of the rat brain are being mapped for localization of noradrenergic and cholinergic terminal innervation.

(4) Several unrelated studies involving the growth of nerves in organ and dissociated cell cultures have been pursued. It has been demonstrated that ganglia from one species can reinnervate the iris of another species.

It was also shown that explants of the substantia nigra of the newborn rat brain are capable of growth of dopaminergic nerve terminals for up to six days.

Dissociated chormaffin cells from sympathetic fetal ganglia have the ability to produce extensive ramifications of varicose terminal catecholamine-containing processes. It is believed that chromaffin cells may play an active role in ganglionic function.

Studies of various clones of mouse neuroblastoma cells have revealed a few clones with scattered intense fluorescent catecholamine-containing cells, thereby demonstrating the potential capability of these cells to differentiate.

(5) Degeneration and regeneration is being studied in vivo in peripheral nerves by the use of vinblastine, a drug which has been shown to inhibit axoplasmic flow. Vinblastine placed directly on the superior cervical ganglion causes degeneration of both the postganglionic sympathetic trunks and the preganglionic cholinergic nerve endings. This initiates an enormous response of sprouting of catecholamine-containing terminals in the ganglion. Thus, catecholamine terminals emanate from adrenergic neurons and/or chromaffin cells within the ganglion. The mechanism which initiates sprouting of new nerves is a problem of major interest.

(6) Since vinblastine was demonstrated to destroy cholinergic preganglionic nerves, it was of interest to use it as a tool to destroy cholinergic (vagal) nerves in the dog heart. This study has led to the observation that there is a rich network of cholinergic nerves intimately related to Purkinje fibers in the ventricular septum and that stimulation of the vagus nerve decreases vulnerability of the ventricle to current-produced fibrillation.

(7) An intraventricular injection of 5,6-dihydroxytryptamine induced a reduction of serotonin concentrations in various parts of the rat brain. These changes in brain serotonin content were not affected by either pretreatment or subsequent treatment with morphine pellets. Naloxone-induced abstinence syndrome was not modified by 5,6-dihydroxytryptamine, although the serotonin content in

Thoa, N.B., Eichelman, B., Richardson, J.S. and Jacobowitz, D.: 6-Hydroxydopa depletion of brain norepinephrine and the facilitation of aggressive behavior. Science 178: 75-77, 1972.

Patil, P.N., Fudge, K. and Jacobowitz, D.: Steric aspects of adrenergic drugs XVIII. Alpha-adrenergic receptors of mammalian aorta. Eur. J. Pharmacol. 19: 79-87, 1972.

Ciaranello, R.D., Jacobowitz, D. and Axelrod, J.: Effect of dexamethasone on phenylethanolamine-N-methyltransferase in neonatal rat chromaffin tissue. J. Neurochem. 20: 799-807, 1973.

Jacobowitz, D.: Distribution of biogenic amines in the pituitary gland. Prog. Brain Res., in press.

Richardson, J.S. and Jacobowitz, D.M.: Depletion of brain norepinephrine by intraventricular injection of 6-hydroxydopa: A biochemical, histochemical and behavioral study in rats. Brain Res., in press.

Balch, T.S., Chase, T.N. and Jacobowitz, D.M.: Dopamine histofluorescence: Restoration in mammalian brain tissues prepared several hours postmortem. Brain Res., in press.

Coyle, J.T., Jacobowitz, D., Klein, D. and Axelrod, J.: Dopaminergic neurons in explants of substantia nigra in culture. J. Neurobiol., in press.

Serial No. M-CS-OC(C)-21
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The study of cerebral catecholamine metabolism in man

Previous Serial Number: None

Principal Investigators: Thomas N. Chase, Michael H. Ebert, Frederick K. Goodwin, Irwin J. Kopin and Robert M. Post (Guest Worker).

Cooperating Units: Sections on Psychiatry and Experimental Therapeutics, Office of the Chief, LCS.

Man Years:		
	Total	3.0
	Professional	2.0
	Other	1.0

Project Description:

Objectives: To study the metabolites of norepinephrine and dopamine in cerebrospinal fluid and other biological fluids and tissues. To correlate changes in concentration or relative amounts of various metabolites, with changes in behavior, affective states or neurologic disorders. To use this information to get insight into basic knowledge of production, turnover and transport of norepinephrine and dopamine in human brain.

Methods Employed: Fluorimetric and gas chromatographic assays are done for some of the studies. Methods are now developed for the assay of neutral and acid metabolites by a combination of gas chromatography- mass spectrometry using duterated tracers. Dihydroxy compounds are studied by enzymatic methylation with radioactive tracer and subsequent radioactive scintillation counting.

Major Findings: A considerable part of the work of the Unit of Clinical Biochemistry was done in collaboration with the Section on Psychiatry.

Human urine which reflects a sum of peripheral plus central nervous system metabolism contains more vanillyl mandelic acid (VMA) than 3-methoxyphenyl glycol (MHPG). Human CSF contains higher concentrations of MHPG than VMA. Since MHPG has been shown to be the main metabolite of animal brain these findings suggest that the norepinephrine metabolites found in human CSF reflect brain metabolism.

It was found that MHPG sulfate did not increase in human lumbar CSF after probenecid treatment suggesting that lumbar CSF may not reflect transport of this metabolite out of the brain - as has been shown in animals by other laboratories. The mechanism of this will be further investigated.

The analysis of CSF of a number of depressed, manic and neurologic patients for MHPG has confirmed the earlier finding of this Unit that the CSF of depressed patients has significantly lower concentration of this norepinephrine metabolites than does the CSF of other patient groups.

Significance to Biomedical Research and the Progress of the Institute: Analysis of biochemical pathways functioning in patients and comparisons of similarities or differences in various affective or pathologic states is a means of associating biochemical peculiarities with abnormal mental states. Changes in metabolic pathways caused by drugs can also be studied. This information is of value in gaining insight into the interrelation of biochemistry and behavior.

Proposed Course of Project: In the past, information has been gained from the analyses of urine. More recently, we have been able to assay a single dopamine (homovanillic acid (HVA) metabolite and a single norepinephrine metabolite (MHPA) in cerebrospinal fluid. Only relatively large concentrations or changes in concentrations could be measured by these procedures. The extension of these studies to include other metabolites and the more sensitive and more specific assays possible with the gas chromatography - mass spectrometry technique will allow us to gain more insight into alterations or aberations of human central nervous system metabolism.

Honors and Awards: None

Publications:

Gordon, E. K., Oliver, J., Goodwin, J.K., Chase, T.N. and Post, R.M.: Effect of probenecid on free 3-methoxy-4-hydroxyphenylthyline glycol (MHPG) and its sulfate in human cerebrospinal fluid. Neuropharmacology, 12: 391-396, 1973.

Post, R.M., Goodwin, F.K., Gordon, E. and Watkins, Donald M.: Amine metabolites in human cerebrospinal fluid: effects of cord transition and spinal fluid block. Science 179: 897-899, 1973.

Post, R.M., Gordon, E.K., Goodwin, F.K. and Bunney, W.E., Jr.: Central norepinephrine metabolism in affective illness: 3-methoxy-4-hydroxyphenylglycol (MHPG) in cerebrospinal fluid. Science, 179: 1002-1003, 1973.

Goodwin, F.K., Post, R.M., Dunner, D.L. and Gordon, E.K.: Cerebrospinal fluid amine metabolites in affective illness: the probenecid technique. Amer. J. Psychiat. 130: 73-79, 1973.

Post, R.M., Kotin, J., Goodwin, F.K. and Gordon, E.K.: Psychomotor activity and cerebrospinal fluid amine metabolites in affective illness. Amer. J. Psychiat. 130: 67-72, 1973.

Mendell, J.R., Murphy, D.L., Engel, W.K., Chase, T.N. and Gordon, E.K.: Catecholamines and indoleamines in patients with duehenne muscular dystrophy. Arch. Neurol. 27: 518-520, 1972.

Serial No. M-CS-M(C)-08
1. Laboratory of Clinical Science
2. Section on Medicine
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Formation, release, disposition and metabolism of biogenic amines

Previous Serial Number: Same

Principal Investigator: Irwin J. Kopin

Other Investigators: Robert W. Colburn, Andre Dubois (Guest Worker), Michael H. Ebert, Ingeborg Hanbauer, David P. Henry, J. Stephen Kizer, Friedhelm Lamprecht, Gertrude D. Maengwyn-Davies (Guest Worker), Jonathan Moss (Guest Worker), L.K.Y. Ng (Guest Worker), Nguyen B. Thoa (Guest Worker), James A. Thomas, Virginia K. Weise

Cooperating Units: Section on Experimental Therapeutics, LCS and Units on Psychosomatics and Analytical Biochemistry (Office of the Chief), LCS

Man Years:		
	Total	7.5
	Professional	6.0
	Other	1.5

Project Description:

Objectives: (1) To determine the mechanisms which control development and plasticity of the nervous system. (2) To study the molecular basis for control of synthesis, storage, release, action and inactivation of aminergic transmitters. (3) To quantify metabolic rates for neurotransmitters and correlate them with function, drug effects and environmental influences.

Methods Employed: Radioactive amines or their precursors are injected into animals, perfused through tissues or exposed to tissue slices *in vitro*; and the metabolism and disposition of the compounds are examined. Enzymes concerned with synthesis or metabolism are assayed in various tissues; and the effects of drugs, procedures, age, etc., are determined.

Major Findings: The elevation of adrenal medullary enzymes results from repeated exposure to stress. Immobilization stress results in elevation of endogenous norepinephrine, increased fighting behavior and increased blood pressure. Serum dopamine-beta-hydroxylase levels are increased during immobilization but are decreased with development of several forms of experimental hypertension in rats and may be an index of the onset of blood pressure elevation in man. The response of adrenal medullary enzymes to the stress of fighting in mice appears to be related to being attacked rather than to attacking. The stress response can be prevented or diminished by treatment with phenobarbital or other psychoactive drugs. Stimulation of sympathetic nerves results in release of dopamine-beta-hydroxylase as well as norepinephrine; and the enzyme can be used to study the process of exocytosis, which mediates release of the neurotransmitter. In the guinea pig vas deferens, the process of transmitter and enzyme release is enhanced by calcium ions. Dibutyryl cyclic-AMP or theophyllin, presumably acting by elevating tissue levels of cyclic-AMP, enhance release of dopamine-beta-hydroxylase and norepinephrine. They may act by mobilizing intracellular calcium. Tyramine releases norepinephrine, but not dopamine-beta-hydroxylase, by a process which is not dependent on calcium and probably does not involve exocytosis. Scorpion venom contains a toxin which causes a calcium-dependent exocytotic release of norepinephrine and dopamine-beta-hydroxylase. The actions of the toxin are prevented by tetrodotoxin and by desmethylimipramine.

Colchicine and vinblastine, by interacting with neurotubulin, block rapid axonal transport. These drugs, when applied to the superior cervical ganglion of rats, cause elevation in levels of catecholamines and of dopamine-beta-hydroxylase. After the initial increase in levels of the enzyme, the amount of dopamine-beta-hydroxylase present diminishes. Postganglionic section or treatment with 6-hydroxydopamine, which destroys sympathetic nerve endings, results in a similar decline in dopamine-beta-hydroxylase activity with a similar decline in levels of tyrosine hydroxylase. Uptake of norepinephrine by the ganglia in vitro, however, increases at the time that dopamine-beta-hydroxylase and tyrosine hydroxylase fall. These observations suggest that, as a result of axonal injury, there has been a switch in metabolism in the ganglia which results in decreased enzyme production and enhanced formation of structural components.

Gastrointestinal paralysis following abdominal surgery appears to be releated to adrenergic discharge in the smooth muscle of the intestine. In rats, this surgical complication can be reduced or eliminated by pretreatment with bretylium.

After injection of 6-hydroxydopamine into rat brain, there are changes in endocrine function which suggest that catecholamine neurotransmitters in the hypothalamus influence release of chemical factors controlling pituitary function.

Significance to Biomedical Research and the Program of the Institute: Catecholamines and other amines play an important role in normal physiology. Alterations in synthesis, activation, inactivation or metabolism of these substances may be responsible for abnormal clinical states or may explain the mode of action of drugs and hormones or development of tolerance or addiction to drugs. Assessment of these various processes is important in determining the factors influencing physiology, pharmacology and pathology in brain and other organs.

Proposed Course of Project: Continued development of methods to study control and to quantify synthesis rates, storage, release and metabolic routes of the catecholamines in isolated tissues, intact animals and man and the study of these mechanisms of neurohumor inactivation in various clinical states of autonomic and mental disorders will be pursued. Assessment of the control of enzymes concerned with transmitter synthesis and their roles in control of neuronal and endocrine function will be further studied.

Honors and Awards: (1) Invited to participate in development of a monograph on "The Catecholamines, Their Function and Dysfunction" for Health Learning Systems. (2) Visiting Scholar-Lecturer at Michigan State University on "How drugs act at adrenergic nerve endings". (3) Invited to give lecture on "Control of catecholamine-biosynthetic enzymes" at Temple University, Department of Pharmacology. (4) Invited to give lecture on "Regulation of catecholamine-synthetic enzymes" at Conference on Some Newer Concepts in Neurotransmitter Regulation in San Diego, California (July 22-23, 1972). (5) Invited to moderate a session on "Special Problems in Pharmacology" at the Fifth International Congress on Pharmacology in San Francisco, California (July 23-28, 1972). (6) Invited to present paper on "Drugs that act at sympathetic nerve endings" to Intra-Science Research Foundation Symposium in Santa Monica, California (November 30-December 2, 1972).

Publications:

Dubois, A., Watanabe, A.M. and Kopin, I.J.: Postoperative gastric ileus. Amer. J. Dig. Dis. 18: 39-42, 1973.

Kvetnansky, R. and Kopin, I.J.: Activity of adrenal catecholamine-producing enzymes and their regulation after stress. In Kovach, A.G.B. (Ed.): Advances in Experimental Medicine and Biology, Volume 33. New York: Plenum Publishing Corporation, 1973 (pp. 517-533).

Lamprecht, F., Eichelman, B.S., Thoa, N.B., Williams, R.B. and Kopin, I.J.: Rat fighting behavior: Serum dopamine-beta-hydroxylase and hypothalamic tyrosine hydroxylase. Science 177: 1214-1215, 1972.

Lamprecht, F., Williams, R.B. and Kopin, I.J.: Serum dopamine-beta-hydroxylase during development of immobilization-induced hypertension. Endocrinology 92: 953-956, 1973.

Maengwyn-Davies, G.D., Johnson, D.G., Thoa, N.B., Weise, V.K. and Kopin, I.J.: Influence of isolation and of fighting on adrenal tyrosine hydroxylase and phenylethanolamine-N-methyltransferase activities in three strains of mice. Psychopharmacologia 28: 339-350, 1973.

Murphy, D.L. and Kopin, I.J.: The transport of biogenic amines. In Hokin, L.E. (Ed.): Metabolic Pathways, Volume VI. New York: Academic Press, Inc., 1972 (pp. 503-542).

Dubois, A., Weise, V.K. and Kopin, I.J.: Postoperative ileus in rat: Physiopathology, etiology and treatment. Ann. Surgery, in press.

Hanbauer, I., Kopin, I.J., Maengwyn-Davies, G.D., Thoa, N.B. and Weise, V.K.: Effect of vinblastine on catecholamine-biosynthetic enzymes in heart, sympathetic ganglion and adrenal glands of rats. Brit. J. Pharmacol., in press.

Lamprecht, F. and Wooten, G.F.: Effect of hypophysectomy on serum dopamine-beta-hydroxylase activity in rat. Endocrinology, in press.

Wooten, G.F., Thoa, N.B., Kopin, I.J. and Axelrod, J.: Enhanced release of dopamine-beta-hydroxylase and norepinephrine from sympathetic nerves by dibutyryl cyclic-AMP and theophyllin. Molec. Pharmacol., in press.

Serial No. M-CS-M(C)-11
1. Laboratory of Clinical Science
2. Section on Medicine
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: False neurochemical transmitters

Previous Serial Number: Same

Principal Investigator: Irwin J. Kopin

Other Investigators: L.K.Y. Ng (Guest Worker), Nguyen B. Thoa (Guest Worker), and Virginia K. Weise

Cooperating Units: Unit on Psychosomatics (Office of the Chief) and Section on Experimental Therapeutics, LCS

Man Years:		
	Total	1.5
	Professional	1.0
	Other	0.5

Project Description:

Objectives: To determine the consequences of neurochemical transmitter replacement by relatively inactive substances, "false transmitters," in the mechanism of action of drugs, to study the possible role of these substitute transmitters in disease states and to investigate the mechanism of transduction of the nerve impulse into transmitter release.

Methods Employed: (1) Radioactively labeled substances which are known or thought to accumulate in sympathetic nerves are synthesized and purified. (2) The effect of precursors of false transmitters on the rate of synthesis of catecholamines is determined using conversion of labeled tyrosine to octopamine and norepinephrine. (3) The central effects of L-dopa have been studied in animals in which the adrenergic and dopaminergic neurons have been destroyed with 6-hydroxydopamine. This permits study of uptake and metabolism of dopa and dopamine in serotonergic neurons.

Major Findings: Amines related to norepinephrine can replace it at its binding sites and thereby influence norepinephrine synthesis, storage and release. The effects depend on the time after administration. Dopamine, formed from dopa, in serotonergic neurons can displace serotonin and diminish synthesis and release of this indoleamine. Dopamine may be a false serotonergic transmitter since it can be released by 5-hydroxytryptophan. This process occurs in brain and can be demonstrated in synaptosomes as well as in peripheral neurones.

Significance to Biomedical Research and the Program of the Institute: An understanding of the mechanisms for binding and release of catecholamines is basic to a meaningful hypothesis of the events related to the activation of transmitters and synaptic transmission. The fact that a number of inactive substances chemically related to norepinephrine may replace it provides a means for modulating the effects of nerve stimulation. It is possible that after drug administration or in disease states, endogenously formed false transmitters may reduce effectiveness of neurotransmission through certain nervous pathways. After administration of L-dopa, accumulation of dopamine and replacement of other amines in brain may result in hypotension and movement disorders.

Proposed Course of the Project: Further investigations of replacement by false transmitters of other neurotransmitters such as cholinergic or serotonergic false transmitters are planned. The possible existence of other molecular substitutes (hormones, for example) will also be investigated. Studies of the possible role of false transmitters in drug action in man are planned.

Honors and Awards: Dr. Kopin presented a lecture on Modification by inhibition of decarboxylase of the cardiovascular effects of L-dopa in animals to the Parkinson's Disease Foundation Symposium, Columbia University, New York on November 28, 1972.

Publications:

Kopin, I.J.: Aminergic neurons and substitute transmitters in the brain. In Kopin, I.J. (Ed.): Research Publications: Association for Research in Nervous and Mental Disease, Volume 50 (Neurotransmitters). Baltimore: Williams and Wilkins Company, pp. 207-215, 1972.

Kopin, I.J.: Modification by inhibition of decarboxylase of the cardiovascular effects of L-dopa in animals. In Yahr, M.D. (Ed.): Advances in Neurology, Volume 2 (Treatment of Parkinsonism - The Role of Dopa Decarboxylase Inhibitors. New York: Raven Press, pp. 137-148, 1973.

Ng, L.K.Y.; Chase, T.N.; Colburn, R.W. and Kopin, I.J.: L-dopa in parkinsonism: A possible mechanism of action. Neurology 22: 688-696, 1972.

Ng, L.K.Y.; Chase, T.N.; Colburn, R.W. and Kopin, I.J.: Release of ^{3}H-dopamine by L-5-hydroxytryptophan. Brain Res. 45: 499-505, 1972.

Ng, L.K.Y.; Colburn, R.W. and Kopin, I.J.: Effect of L-dopa on accumulation and efflux of monoamines in particles of rat brain homogenates. J. Pharmac. Exp. Ther. 183: 316-325, 1972.

Thoa, N.B.; Weise, V.K. and Kopin, I.J.: Effect of L-dihydroxyphenylalanine on methylation of ^{3}H-norepinephrine and ^{3}H-histamine. Biochem. Pharmac. 21: 2345-2350, 1972.

Serial No. M-CS-M(C)-12
1. Laboratory of Clinical Science
2. Section on Medicine
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Growth characteristics of aminergic neurons

Previous Serial Number: None

Principal Investigator: Irwin J. Kopin

Other Investigators: Julius Axelrod, Kenneth R. Berv, Floyd E. Bloom, Ingeborg Hanbauer, David M. Jacobowitz, Jerry G. Webb, J. Stephen Kizer

Cooperating Units: Laboratory of Neuropharmacology, NIMH; Unit on Histopharmacology (Office of the Chief) and Section on Pharmacology, LCS

Man Years:		
	Total	4.5
	Professional	4.0
	Other	0.5

Project Description:

Objectives: The development of in vitro techniques to study tissues in isolation permits examination of factors which control processes of growth and influence levels of function of neurons.

Methods Employed: Tissues are removed from animals and transferred to vessels where they are maintained in culture and allowed to grow. The constituents of the culture media are varied; different tissues such as superior cervical ganglia and irises can be grown together, and drugs and hormones can be added to the media. With such in vitro techniques, the rate of change in enzyme levels, amine content and uptake processes can be measured, and the effects of various factors on growth of axonal processes can be assessed.

Major Findings: Adrenal glands, superior cervical ganglia, irises and pineal glands from adult rats survive for up to two weeks in organ culture. When ganglia are grown in contact with irises or pineal glands, axonal processes grow into tissue. The reinnervation process is not specific for tissues of the same species since rat ganglia can innervate mouse iris. The axonal sprouts enter tissues and carry with them vesicles containing dopamine-beta-hydroxylase and catecholamines.

Conditions which enhance release of catecholamines (e.g. depolarization induced by elevated potassium) cause induction of tyrosine hydroxylase and dopamine-beta-hydroxylase in adrenal glands and ganglia.

Pituitary glands incubated with small fragments of hypothalamic nuclei show enhanced or diminished release of prolactin or other hormones. These recently begun studies promise to yield information on the control of hypothalamic-releasing factors.

Significance to Biomedical Research and the Program of the Institute: Understanding the factors which ocntrol neuronal growth and development is essential for a rational approach to investigation of a wide spectrum of physiologic processes (e.g. memory, maturation, endocrine control) and pathologic conditions (neural retardation, drug influences on brain development and perhaps a number of neurologic and psychiatric disorders). The study of neurons in isolated systems permits controlled variations of the cellular environment, which are not possible in the intact animal, and thus allows more definitive identification of the factors involved in control of the various functions.

Proposed Course of Project: Tissue from brain and peripheral sympathetic neurons will be grown. The changes in uptake processes, enzyme levels, neurotransmitter formation and release and the effects of other cells will be examined. The rate of hormones, the effects of drugs and the differences in genetic variants will be investigated.

Honors and Awards: Invited to present a lecture on "Sympathetic ganglia in organ culture" at the Third International Catecholamine Symposium to be held in Strasbourg, France (May 20-25, 1973).

Publications:

Hanbauer, I., Johnson, D.G., Silberstein, S.D. and Kopin, I.J.: Pharmacological and kinetic properties of uptake of ^{3}H-norepinephrine by superior cervical ganglia of rats in organ culture. Neuropharmacology 11: 857-862, 1972.

Johnson, D.G., Silberstein. S.D., Hanbauer, I. and Kopin, I.J.: The role of nerve growth factor in the ramification of sympathetic nerve fibres into the rat iris in organ culture. J. Neurochem. 19: 2025-2029, 1972.

Kopin, I.J., Silberstein, S.D., Johnson, D.G., Hanbauer, I. and Jacobowitz, D.M.: Sympathetic reinnervation ofhe rat iris in vitro. In Costa, E., Iversen, L.L. and Paoletti, R. (Eds.): Advances in Biochemical Psychopharmacology, Volume 6. New York: Raven Press, 1972 (pp. 89-92).

Silberstein, S.D., Lemberger, L., Klein, D.C., Axelrod, J. and Kopin, I.J.: Induction of adrenal tyrosine hydroxylase in organ culture. Neuropharmacology 11: 721-726. 1972.

Vogel, S.A., Silberstein, S.D., Berv, K.R. and Kopin, I.J.: Stimulation-induced release of norepinephrine from rat superior cervical ganglia in vitro. Eur. J. Pharmacol. 20: 308-311, 1972.

Cramer, H., Johnson, D.G., Hanbauer, I., Silberstein, S.D. and Kopin, I.J.: Accumulation of adenosine-3',5'-monophosphate induced by catecholamines in the rat superior cervical ganglia in vitro. Brain Res., in press.

Johnson, D.G., Weise, V.K., Hanbauer, I., Silberstein, S.D. and Kopin, I.J.: Dopamine-beta-hydroxylase activity during sympathetic reinnervation of rat iris in organ culture. Neurobiology, in press.

Kopin, I.J., Berv, K.R. and Webb, J.G.: Organ culture of sympathetic ganglia. In *Third International Pharmacology Symposium*. New York: Pergamon Press, 1973 (in press).

Serial No. M-CS-Ps(C)-19
1. Laboratory of Clinical Science
2. Section on Psychiatry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The use of the cerebrospinal fluid for the direct assessment of neurochemical systems in man.

Previous Serial Number: None

Principal Investigator: Frederick K. Goodwin

Other Investigators: Robert M. Post, Edna K. Gordon

Other Collaborating Scientists: Dennis L. Murphy, Michael H. Ebert, Irwin J. Kopin

Cooperating Units: 4-West and 3-East Clinical Research Units, LCS
Unit on Clinical Biochemistry, LCS

Man Years:		
	Total	3.4
	Professional	2.7
	Other	0.7

Project Description:

Objectives: The objective of this project is the continued development of methods for the estimation of neurochemical alterations in patients through the use of the cerebrospinal fluid (CSF). The objectives include a full exploration of both the potential and the limitations of the CSF as a "window into the brain".

Methods Employed:

Sampling of the CSF is accomplished by lumbar puncture. The position of the patient, time of day, and antecedent activity have all been examined for a possible effect on the metabolite levels under study. Headache, the major side effect of lumbar puncture, has been considerably reduced by alteration of the conditions that we have found to contribute to headache. As an adjunct to the CSF studies, the drug probenecid is employed. Probenecid inhibits the active transport of acid metabolites of brain amines, 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA), the major breakdown products of serotonin and dopamine, respectively. The CSF levels of these acid metabolites are closely related to brain levels and their rate of accumulation during probenecid administration can be used as an indirect index of amine turnover.

In collaboration with the Unit on Clinical Biochemistry (Mrs. Gordon), CSF amine metabolite assays employing gas-liquid chromatography and mass spectroscopy are under continued development.

Major Findings

(1) Data on baseline levels of amine metabolites in the CSF of 70 patients with affective illness has been analyzed to uncover possible relationships to diagnostic subtypes, severity of symptoms, types of symptoms (such as agitation vs. retardation), subsequent drug response and other clinical features. Of the various clinical parameters examined, only the psychomotor agitation-retardation continuum has been found to correlate with levels of the metabolites of dopamine (HVA) and serotonin (5HIAA). Extension of the studies comparing depressed, manic and control populations indicate no differences in 5HIAA levels, a reduction in both HVA and MHPG (a norepinephrine metabolite) in depressed patients compared to controls or manic patients.

(2) CSF amine metabolite levels in patients with cord transection and spinal fluid block: These studies, under the direction of Dr. Robert Post, were initiated in order to answer questions concerning the origin of amine metabolites in the lumbar CSF of man. Patients with traumatic injury to the spinal cord were selected from the Neurology Services of two V.A. Hospitals and studied with lumbar puncture. Results indicate an absence of HVA in those patients with blockade of CSF flow and the persistence of some 5HIAA and MHPG in the presence of complete cord transection, regardless of the presence or absence of spinal fluid block. These data suggest some local (cord) contribution to the lumbar CSF levels of 5HIAA and MHPG, but not HVA.

(3) The effect of simulated behavioral states on CSF amine metabolites: These studies, initiated last year, have been extended, particularly to evaluate the relationship between activity related alterations in amine metabolites in CSF and similar changes in urinary metabolites. Significant increases in HVA, 5HIAA and MHPG in the CSF have been found to occur when moderately depressed patients simulate the hyperactivity of mania.

(4) CSF amine metabolites in affective illness: Studies with Probenecid: The further development and application of the probenecid technique has been a major research focus over the past year. A number of clinically relevant differences in central nervous system amine dynamics have been found by this technique -- in other words, uncovering differences that were not reflected in CSF amine metabolite levels alone. Specifically, a greater accumulation of HVA on probenecid (probably reflecting more rapid central dopamine turnover) has been found to differentiate bipolar from unipolar depressed patients. The extent of accumulation of both 5HIAA and HVA on probenecid has some value in predicting subsequent antidepressant response to lithium, but not to tricyclic antidepressants. Finally, we have found a significant decrease in the probenecid-induced accumulation of 5HIAA following three separate antidepressant treatments -- electroconvulsive therapy, tricyclics and lithium.

(5) A system in primates for the chronic simultaneous sampling of ventricular and lumbar CSF: Under the leadership of Dr. Post, and in collaboration with Dr. Ebert, a major effort has been initiated this year towards the development of a reliable system for the simultaneous collection of ventricular and lumbar CSF from living monkeys. Employing I^{131}, considerable data relating to the dynamics of CSF flow and to the location of the metabolite transport systems has been gained. The accumulation of acid metabolites following probenecid has been demonstrated. It has been shown that it is possible to maintain healthy monkeys for several weeks with chronic collection systems in place.

Publications:

Cramer, H., Goodwin, F.K., Post, R.M., and Bunney, W.E., Jr.: Effect of probenecid and exercise on cerebrospinal fluid cyclic AMP in affective illness. Lancet, ii:1346-1347, 1972.

Gordon, E.K., Oliver, J., Goodwin, F.K., Chase, T.M., and Post, R.M.: Effect of probenecid on free 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and its sulfate in human cerebrospinal fluid. Neuropharmacol. 12:391-396, 1973.

Post, R.M., Gordon, E.K., Goodwin, F.K., and Bunney, W.E., Jr.: Central norepinephrine metabolism in affective illness: MHPG in the cerebrospinal fluid. Science, 179:1002-1003, 1973.

Post, R.M., Goodwin, F.K., Gordon, E., and Watkin, D.M.: Amine metabolites in human cerebrospinal fluid: effects of cord transection and spinal fluid block. Science, 179:897-899, 1973.

Goodwin, F.K., Post, R.M., Dunner, D.L., and Gordon, E.K.: Cerebrospinal fluid amine metabolites in affective illness: the probenecid technique. Amer. J. Psychiat., 130:73-79, 1973.

HONORS AND AWARDS

Dr. Goodwin: See project report #M-CS-PS(C)-18

Dr. Post received the A.E. Bennett award from the Society of Biological Psychiatry for his paper (co-authored with Dr. Goodwin) on "Simulated behavior states: an approach to specificity in psychobiological research". Dr. Post was a guest speaker at the Washington Hospital Center, Department of Psychiatry, and presented a paper at the Yale University, Department of Psychiatry on "CSF Amine Metabolites in Affective Illness". Dr. Post will also present a paper on "The Effects of Cocaine in Depressed Patients" at the Annual Meeting of the American Psychiatric Association in Honolulu, Hawaii.

Serial No. M-CS-PS(C)-20
1. Laboratory of Clinical Science
2. Section on Psychiatry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biogenic amine-related enzymes and transport mechanisms in human cells

Previous Serial Number: None

Principal Investigator: Dennis L. Murphy

Collaborating Scientists: Robert Belmaker, Thomas Chase, W. King Engel, Ira Lott, Jerry Mendell, William Pollin, Richard W. Wyatt

Man Years:		
	Total	2.0
	Professional	0.4
	Other	1.6

Project Description:

Objectives: This study was initiated to develop techniques for the study of biogenic amine metabolism in man at the cellular level.

Methods Employed: Platelets contain amine storage vesicles, mitochondria with monoamine oxidase activity and have a membrane transport mechanism for serotonin which is kinetically similar to that found in brain. Drugs such as the tricyclic antidepressants, reserpine and lithium have similar effects on platelets and neurons, and thus human platelets were chosen as a model cellular system for the investigation of transport, storage, release and enzymatic degradation of biogenic amines in different psychiatric and neurologic disorders and in relation to individual differences in psychoactive drug effects.

Major Findings: Monoamine oxidase (MAO) activity in human platelets has been found to be a relatively stable characteristic of individuals which is markedly affected by MAO-inhibiting antidepressants but not by other drugs or environmental conditions. Studies comparing normal monozygotic and dizygotic twins suggest that most of the individual variation in platelet MAO is genetically determined. In different psychiatric patients, MAO activity has been found to be no different from normal controls in unipolar depressed patients, but moderately reduced in bipolar depressed patients and more severely reduced in schizophrenic individuals. In monozygotic twins discordant for schizophrenia, both twins exhibit reduced activity, suggesting that the enzyme difference is not a result of the chronic disorder or other events related to it, such as phenothiazine treatment, but rather may prove to be a genetic marker for vulnerability to this disorder.

Serotonin levels in platelets have been found to be increased by L-tryptophan treatment, and decreased by L-dopa administration to patients. In vitro explorations of the mechanism of the L-dopa effect indicate that dopamine and other catecholamines both competitively inhibit ^{14}C-serotonin accumulation in cells and also act to directly displace serotonin from platelets, suggesting a potential for "false transmitter" type effects during amine precursor administration in man.

Techniques for the study of platelet amine mechanisms developed in this laboratory were extended to study suggested serotonin-related processes in children with Down's syndrome and muscular dystrophy, Markedly reduced platelet serotonin levels previously reported in Down's syndrome were confirmed, and studies of potential mechanisms for this change indicated that serotonin transport into platelets was reduced; cerebrospinal fluid studies did not indicate that similar changes in serotonin transport or metabolism occur in the central nervous system. While children with muscular dystrophy were found to have normal urinary excretion of indoleamine and catecholamine metabolites, some platelet transport alterations in these patients were observed and are consistent with an experimental model implicating microvascular regulatory abnormalities in this disorder.

Significance to Biomedical Research and the Program of the Institute: These studies indicate the feasibility of methods other than measurement of final amine metabolite levels in urine and CSF for the study of biogenic amine biochemistry in man. The observed differences in biogenic amine-related enzymes and transport processes in individuals with different psychiatric and neurologic disorders may be relevant to some features of these disorders.

Proposed Course of the Project: Laboratory and clinical studies of platelet monoamine oxidase will seek to determine the relationship of this enzyme to the various MAO isoenzymes in brain, and to determine by studying other populations under different environmental conditions the specificity of the changes observed in the present studies. Further studies of platelet mechanisms including amine receptor functions will be directed towards the evaluation of this system for the study of individual differences in drug effects in man.

Honors and Awards:

Dr. Murphy discussed "Differences in biogenic amine metabolism and enzymes affecting biogenic amines in subgroups of patients with psychiatric disorders" at the American College of Neuropsychopharmacology meeting in San Juan, Puerto Rico. He presented a paper entitled "The clinical pharmacology of psychoactive drugs affecting biogenic amine transport, storage and receptor functions," at the Mayo Clinic Medical School, Rochester. At the Third International Catecholamine Symposium in Strasbourg, France, he was invited to discuss "Technical Strategies for the Study of Catecholamines in Man" and at an associated meeting on Catecholamines and Schizophrenia in Strasbourg he presented a paper on "Clinical and Laboratory Studies of Human Platelet Monoamine Oxidase."

Publications:

Murphy, D.L. and Weiss, R.: Reduced monoamine oxidase activity in blood platelets from bipolar depressed patients. Amer. J. Psychiat. 128:1351-1357, 1972.

Murphy, D.L. and Wyatt, R.J.: Reduced monoamine oxidase activity in blood platelets from schizophrenic patients. Nature 238:225-226, 1972.

Wyatt, R.J., Murphy, D.L., Belmaker, R., Cohen, S., Donnelly, C.H. and Pollin, W.: Reduced monoamine oxidase activity in platelets: A possible genetic marker for vulnerability to schizophrenia. Science 179:916-918, 1973.

Murphy, D.L.: Amine precursors, amines and false neurotransmitters in depressed patients. Amer. J. Psychiat. 129:141-148, 1972.

Lott, I.T., Chase, T.H. and Murphy, D.L.: Down's syndrome: transport, storage and metabolism of serotonin in blood platelets. Pediat. Res. 6:730-735, 1972.

Lott, I.T., Murphy, D.L. and Chase, T.H.: Down's Syndrome: central monoamine turnover in patients with diminished platelet serotonin. Neurology 22:967-972, 1972.

Mendell, J.R., Murphy, D.L., Engel, W.K., Chase, T.N. and Gordon, E.: Catecholamines and indoleamines in patients with Duchenne muscular dystropy. Arch. Neurol. 27:518-520, 1972.

Murphy, D.L., Mendell, J.R. and Engel, W.K.: Serotonin and platelet function in patients with Duchenne muscular dystrophy. Arch. Neurol. 28:239-242, 1973.

Murphy, D.L. and Kopin, I.J.: The Transport of Biogenic Amines. In Hokin, L.E. (ed.): Cell Transport and Metabolism. New York, Academic Press, 1972. pp. 503-542.

Serial No. M-CS-PS(C)-22
1. Laboratory of Clinical Science
2. Section of Psychiatry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical phenomenology and psychopharmacology of the affective disorders.

Previous Serial Number: M-CS-PS(C)-18

Co-Principal Investigators: Frederick K. Goodwin, Dennis L. Murphy

Other Investigators: Halbert Miller, Steven Ablon, Eugene Redmond, Laurence Greenwood, Robert Sack, Robert Post (Guest Worker), Eugene Brand (Guest Worker), Daniel van Kammen (Guest Worker) Morris Lipton (Guest Worker)

Collaborating Scientists: Monte S. Buchsbaum, William E. Bunney, Jr., Michael H. Ebert, Edward Donnelly, Herbert Weingartner

Psychiatric Consultants: M.B. Richmond, L. Shaffer, P. Chodoff, M.L. Adland, H.A. Myersberg, J. Boriello

Cooperating Units: Nursing personnel on 3-East and 4-West; Occupational Therapy (Eleanor Stapin, Judith Schreiber) Art Therapy (Sadie Fishman) and Social Work personnel (Yolande Davenport, Carol F. Hoover, Walter Sceery) on Wards 3-East and 4-West

Man Years:		
	Total	11.6
	Professional	6.1
	Other	5.5

Project Description:

Objectives: The objective of these projects is the development of a research program for the investigation of the behavioral, biological and pharmacological aspects of depressed, manic and schizo-affective patients.

Behavioral Objectives: Our behavioral objects are based on the continued development of systems for the quantitative assessment of the clinical phenomenology of affective illness and related psychoses. These phenomenological investigations include the delineation of subgroups whose homogeneity can be validated by clinical, genetic, pharmacologic and biological parameters, and the assessment of the interrelationship between mania, depression and other affective and cognitive states.

Pharmacological Objectives: Studies with established antidepressent or antimanic drugs are directed toward an understanding of their mechanisms of action and toward an understanding of the relationship between drug response and individual differences in clinically or biologically defined patient subgroups.

Additional studies using experimental compounds which are known to affect central amines are used to investigate the relationship between brain biogenic amines and affective illness.

Methods Employed:

1. Manic-depressive and schizo-affective patients are selected for study. The depressed patients demonstrate feelings of hopelessness, thoughts of worthlessness, preoccupation with death and dying, and difficulty sleeping, while the manic patients often show flight of ideas, grandiosity, inappropriate and intrusive behavior and, at times, euphoria. Other patients with psychiatric disorders are also studied because of overlapping characteristics (e.g., schizo-affective patients) or for control and comparison purposes.

2. Behavioral and biochemical data is collected on a longitudinal basis throughout the course of each patient's hospitalization, with behavioral data collected by a nursing research team, social workers, and ward physicians.

a. The nursing research team is trained in systematic methods of observation and recording. At the end of each morning and evening shift, two members of the nursing team independently rate each patient on a 15-point scale. The scale measures the amount of depression, anxiety, psychotic behavior, physical activity, somatic complaints and anger which are manifested during the 8-hour shift. A separate scale has been developed for the measurement of manic symptomatology. The nurses also describe the patients' verbal and non-verbal behavior every eight hours.

b. The staff physicians see each patient in individual psychotherapy two to four times a week. The physicians dictate information following a schedule after each therapy session.

c. The social worker interviews the closest relatives of each patient, seeing them individually on a weekly basis.

d. Observations are made by the nurses concerning the patients' sleep throughout every night. These observations are recorded at half-hour intervals.

Biochemical Data: 24-hour urine pools are collected throughout hospitalization for the determination of urinary catecholamines and indoleamines and their metabolites. Selected samples are also analyzed for drug levels (e.g., lithium) and for electrolyte and steroid studies.

Physiological Data: Selected patients are monitored in terms of their EEG and rapid eye movement potentials throughout each night in collaboration with the staff of the Laboratory of Clinical of Psychobiology. Cortical evoked potentials are studied in some patients in collaboration with Dr. Monte Buchsbaum.

Pharmacological Studies: Active medication and placebo are administered double-blind in a non-random design to many of the patients, with placebo substitution before and after each active compound. The utilization of placebo substitution increased the level of confidence in the efficacy of a compound in an individual patient. This is important in the evaluation of drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness.

Major Findings: The major findings in the Section during the past year are reviewed as follows:

1. Studies of the phenomena of mania have indicated that psychomotor activity and not affect may best characterize the manic state, and that depressive and psychotic elements often are present during different stages of mania. Distinct "stages" of the manic episode have been delineated and specific items have been found to reliably differentiate manic from schizo-affective patients. These findings as well as prior studies of mania and of the bipolar patient group were reviewed by Drs. Murphy, Goodwin and Bunney for a new volume of The American Handbook of Psychiatry.

2. Verbal learning measures studied in depressed patients indicate that depression is associated with diminished longer-term memory which is characterized principally by an increase in errors of omission. These results are in contrast to the manic state, in which a longer-term memory deficit was found to be based on an alteration in the turnover and type of associational structure formed during verbal learning. Spontaneous clinical improvement in depression and improvement resulting from imipramine and lithium produced improvement in learning; L-dopa and L-tryptophan also improved learning, but with these agents the memory improvement was not associated with changes in rated depression. Preliminary studies of state-dependent learning in patients with affective disorders were initiated this year, and suggest that some clinical phenomena of depression and mania may be understood in terms of affective state-dependent recall.

3. Systematic studies of ego defensive patterns in manic-depressive patients have revealed unipolar and bipolar differences as well as similar patterns of change accompanying improvement in either depression or mania.

4. In addition to clinical and genetic differences previously reported, bipolar depressed patients have been found to differ from unipolar patients in their behavioral responsivity to L-tryptophan, in the relative amounts of dopamine and its metabolites excreted after on L-dopa load, and in D, MA, and SI scale scores on the MMPI.

5. Preliminary studies of short-term sleep deprivation have revealed some transient antidepressant effects.

6. Family studies of affective illness have revealed a significant degree of "assortative mating".

7. In a double-blind, randomized-order treatment study of lithium carbonate, phenelzine and imipramine in depressed patients initiated for comparison with studies of experimental drugs in this program, both lithium and imipramine were found to have significant antidepressant effects, while phenelzine was found to be virtually without significant antidepressant action.

8. A newly revised computer-scored affective disorder rating scale has been factor analyzed and has been put into daily use.

Proposed Course of the Project: During the next year continued emphasis will be given to the further development of methods for the study of amine function in man and their application to the study of manic-depressive illness and as a basis for the therapeutic effects of antidepressant and antimanic drugs. Attempts to correlate individual differences in behavioral response to drugs with biochemical changes and with drug metabolism will be continued.

Studies of the natural course of manic-depressive illness will be expanded, using both inpatient and follow-up studies. An attempt will be made to examine psychobiological correlates of the predisposition to affective illness by studying patients with past histories of manic or depressive illness, and their first degree relatives during normal phases.

Family studies focused especially on the genetics of bipolar, schizo-affective and schizophrenic patients will be pursued using monoamine oxidase and other biogenic amine-related enzymes as a major focus of these investigations.

Honors and Awards:

Dr. Murphy discussed "Differences in Biogenic Amine Metabolism and Enzymes Affecting Biogenic Amines in Subgroups of Patients with Psychiatric Disorders" at the American College of Neuropsychopharmacology meeting in San Juan, Puerto Rico. He presented a paper entitled "The Clinical Pharmacology of Psychoactive Drugs Affecting Biogenic Amine Transport, Storage and Receptor Functions," at the Mayo Clinic Medical School, Rochester. At the Third International Catecholamine Symposium in Strasbourg, France, he was invited to discuss "Technical Strategies for the Study of Catecholamines in Man" and at an associated meeting on Catecholamines and Schizophrenia in Strasbourg he presented a paper on "Clinical and Laboratory Studies of Human Platelet Monoamine Oxidase."

Dr. Goodwin was an invited speaker at a Symposium on "Biological Findings in Affective Illness" at the Collegium Internationale Neuropsychopharmacologium (CINP), Copenhagen, Denmark, and at this same meeting he also presented a paper on "The Clinical Effects of Cocaine in Depressed Patients." As an invited speaker at the Western Divisional meeting of the American Psychiatric Association (San Diego, California), he presented a paper on "New Developments in the Treatment of Depression." He also presented a paper entitled "Biological Subtyping of Depression and the Prediction of Antidepressant Response" as part of a symposium at the Annual Meeting of the American College of Neuropsychopharmacology in San Juan, Puerto Rico.

Dr. Goodwin was a guest speaker at the V.A. Collaborative Research meeting in New Orleans at which he presented a paper on "The Unipolar-Bipolar Dichotomy: Implications for the Psychopharmacology of Affective Illness." In addition, Dr. Goodwin was an invited speaker at The Brain Research Institute, University of Tennessee, at the Clark Institute, University of Toronto, at the Case-Western Reserve University School of Medicine and at the University of Munich, Munich, Germany. At the Annual Meeting of the Society of Neuroscience in Houston, Texas, Dr. Goodwin presented a paper on "Cocaine: Clinical Effects in Depressed Patients." At the Annual Meeting of the American Psychiatric Association in Honolulu, Hawaii, Dr. Goodwin will present a paper on "CSF Amine Metabolites and Therapies of Depression" and in addition will be the discussant for a paper on prophylactic lithium; he is also the co-author of three other papers to be presented at this meeting.

Dr. Goodwin will present a paper on "The Catecholamine Hypothesis Revisited" at the International Catecholamine Symposium in Strasbourg, France, and a paper on "The Effects of Antidepressant Treatment on Serotonin Metabolites in the CSF" at the International Serotonin conference in Sardinia, Italy. Dr. Goodwin will present an invited lecture on "Methods for the Study of Amine Turnover in Man" at the International Psychosomatic Congress in Amsterdam, Holland.

Dr. Goodwin was interviewed by Mike Wallace on the CBS program "60 Minutes" as a part of a special program on depression.

Dr. Ablon will present a paper, "Ego Defense Patterns in Manic-Depressive Illness," at the Annual Meeting of the American Psychiatric Association in Honolulu, Hawaii. At the same meeting, in the section for the late breaking research findings, Dr. Ablon will present a paper, "Tetrahydrocannabinol in Depression: High Frequency of Dysphoria."

Dr. Post: See Project Report No. M-CS-PS(C)-19.

Dr. Redmond presented a paper,"Changes in Social Behavior in Monkeys after Inescapable Shock" at the American Psychosomatic Society Meeting in Denver.

Publications:

Murphy, D. L., Goodwin, F.K. and Bunney, W.E., Jr.: Clinical and pharmacological investigations of the psychobiology of the affective disorders. Int. Pharmacopsychiat. 6:137-146, 1971.

Bunney, W.E., Jr., Goodwin, F.K. and Murphy, D.L.: Current Research on L-DOPA in Depression and Mania. In Malitz, S. (ed.): L-DOPA and Behavior. New York, Raven Press, 1971. pp. 87-102.

Murphy, D.L., Goodwin, F.K., Brodie, H.K.H. and Bunney, W.E., Jr.: L-DOPA, dopamine and hypomania. Amer. J. Psychiat. 130:79-82, 1973.

Murphy, D.L., Henry, G.M. and Weingartner, H.: Catecholamines and memory: Enhanced verbal learning during L-DOPA administration. Psychopharmacologia 27:319-326, 1972.

Goodwin, F.K., Murphy, D.L., Dunner, D.L. and Bunney, W.E., Jr.: Lithium response in unipolar versus bipolar depression. Amer. J. Psychiat. 129:44-47, 1972.

Donnelly, E.F., Dent, J.K., Murphy, D.L. and Mignone, R.J.: Comparison of temporal lobe epileptics and affective disorders on the Halstead-Reitan test battery. J. Clin. Psychol. 28:61-62, 1972.

Bunney, W.E., Jr., Murphy, D.L., Goodwin, F.K. and Borge, G.K.: The "switch process" in manic-depressive illness. I. A systematic study of sequential behavior change. Arch. Gen. Psychiat. 27:295-302, 1972.

Bunney, W.E., Jr., Goodwin, F.K., Murphy, D.L. and House, K.M.: The "switch process" in manic-depressive illness. II. Relationship to catecholamines, REM sleep and drugs. Arch. Gen. Psychiat. 27:304-309, 1972.

Bunney, W.E., Jr., Goodwin, F.K. and Murphy, D.L.: The "switch process" in manic-depressive illness. III. Theoretical implications. Arch. Gen. Psychiat. 27:312-317, 1972.

Dunner, D.L., Gershon, E.S., Goodwin, F.K., Murphy, D. L. and Bunney, W.E., Jr.: Excretion of 17-hydroxycorticosteroids in unipolar and bipolar depressed patients. Arch. Gen. Psychiat. 26:360-363, 1972.

Bunney, W.E., Jr., Gershon, E.S., Murphy, D.L. and Goodwin, F.K.: Psychobiological and pharmacological studies of manic-depressive illness. J. Psychiat. Res. 9:207-226, 1972.

Bunney, W.E., Jr. and Murphy, D.L.: The Switch Process and Psychopathology. In Mendels, J. (ed.): Textbook of Biological Psychiatry, New York, Academic Press, Chap. 15, pp. 345-367, 1973.

Goodwin, F.K.: The Psychobiology of Depression: Some Conceptual Issues. In *Psychology of Depression: Contemporary Theory and Research*, Friedman, R. and Katz, M. (eds.), Academic Press, New York, 1972.

Brodie, H.K.H., Murphy, D.L., Goodwin, F.K. and Bunney, W.E., Jr.: Alpha-methyl-para-tyrosine in affective illness. In *Advances in Neuropharmacolgy*, Vinar, O., Vatava, Z. and Bradley, P.B. (eds.), North Holland Publishing Co., Amsterdam, 1971.

Kotin, J., Post, R.M. and Goodwin, F.K.: Δ^9-Tetrahydracannabinol (THC) in depressed patients. *Arch. Gen. Psychiat.* 28:345-348, 1973.

Carlson, G.A. and Goodwin, F.K.: The stages of mania: A longitudinal analysis of the manic episode. *Arch. Gen. Psychiat.* 28:221-228, 1973.

Ebert, M.H., Post, R.M. and Goodwin, F.K.: The effect of physical activity on urinary MHPG excretion in depressed patients. *Lancet* II:766, 1972.

Post. R.M., Kotin, J., Matzen, M., Jones, C.C. and Goodwin, F.K.: Effect of serum from depressed and manic patients on maze behavior of rats. *Experientia* 28:1471, 1972.

Goodwin, F.K. and Bunney, W.E., Jr.: A psychobiological approach to affective illness. *Psychiat. Annals* 3:19-56, 1973.

Murphy, D.L., Goodwin, F.K. and Bunney, W.E., Jr.: A reevaluation of biogenic amines in manic and depressive states. *Hosp. Prac.* 7: 85-96, 1972.

Redmond, D.E., Jr., Maas, J.W., Graham, C.W., and Dekirmenjian, H.: The effect of norepinephrine replenishment on alpha-methyl-p-tyrosine treated monkeys. *Psychosom. Med.* 34:469, 1972.

Serial No. M-CS-Ph-5
1. Laboratory of Clinical Science
2. Section on Pharmacology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemistry and pharmacology of the adrenergic nervous system

Previous Serial Number: Same

Principal Investigators: Roland D. Ciaranello, Joseph T. Coyle, G. Frederick Wooten

Other Investigators: Julius Axelrod

Man Years:		
	Total	5.5
	Professional	3.5
	Other	2.0

Project Description:

Objectives: To study the development of the catecholaminergic nervous system in the brain, in vivo and in organ culture; to examine the mechanisms whereby catecholamine synthesizing enzymes are transported within the axon; to study factors controlling release and maintenance of circulating levels of dopamine-β-hydroxylase in normal and disease states in man; to examine the genetic factors involved in the regulation of catecholamine biosynthetic enzymes.

Methods Employed: Biochemical, pharmacological, immunological and genetic techniques.

Major Findings: We previously have shown that all the enzymes in the biosynthetic pathway for catecholamines are present in the fetal rat brain at 15 days of gestation. In recent studies, dopamine and noradrenaline were found to also be present at this early stage of development; and the levels of the amines increase 15-fold during the one week before birth. The levels of these catecholamines in the fetal brain were found to be under similar controls as that of the adult brain. We have succeeded in maintaining explants of the substantia nigra and corpus striatum of newborn rats in tissue culture. These explants showed outgrowth of dopaminergic neurones which had the same morphologic and pharmacologic properties as normal neurones.

The absolute rate of proximo-distal axoplasmic transport of the enzymes involved in the biosynthesis and metabolism of noradrenaline were established in the sciatic nerve of the rat. Dopamine-β-hydroxylase and tyrosine hydroxylase travel at a very rapid rate; dopa decarboxylase is transported at an intermediate rate, and catechol-O-methyltransferase and monoamine oxidase move at a slow rate down the axon. These studies suggest that tyrosine hydroxylase and dopamine-β-hydroxylase may be closely associated within the neurone. An additional study in the toad has shown that the enzyme that forms adrenaline, phenylethanolamine N-methyltransferase, is transported at a slow rate.

The levels of serum dopamine-β-hydroxylase are related to sympathetic nerve activity in the normal human since various stresses that increase sympathetic tone also increase the enzyme activity in serum. In patients with cervical cord lesions, circulating levels of dopamine-β-hydroxylase increase markedly during hypertensive crises. Patients with the dominant form of familial torsion dystonia have abnormally high levels of serum dopamine-β-hydroxylase, whereas those with the recessive form of the disease have normal levels of the enzyme in their serum. Studies on the exocytotic release of dopamine-β-hydroxylase and noradrenaline as well as on the factors controlling levels of serum dopamine-β-hydroxylase in the rat were carried out in collaboration with the Section on Medicine and will be described by Dr. Kopin.

The genetic factors controlling the levels of the enzymes involved in the synthesis of catecholamines have been examined in the adrenal gland of different strains of mice. The levels of phenylethanolamine N-methyltransferase, the adrenaline-forming enzyme, appear to be controlled primarily by a gene that affects the rate of degradation of the enzyme. Additional studies on immature rats indicate that phenylethanolamine N-methyltransferase can be induced in sympathetic tissue that does not ordinarily synthesize the enzyme by the administration of corticosteroid and ACTH. The induction of the enzyme correlates with the increase in the number of small, intensely fluorescent neurones.

Significance to Biomedical Research and the Program of the Institute: Studies on the development of the adrenergic nervous system in the rat brain may give some insight into the normal development of the brain, the effect of drugs on this process, and the relationship between brain growth and mental retardation. Studies on serum dopamine-β-hydroxylase can provide information concerning the factors involved in controlling the activity of sympathetic neurones in man. In addition, our studies have uncovered an enzymatic marker for distinguishing between two forms of hereditary dystonia.

Proposed Course of Project: Factors involved in axonal transport of enzymes, such as energy requirements, localization of the enzymes within the neurones, and effects of drugs will be examined. Ongoing studies of pharmacological and physiological perturbations on the serum dopamine-β-hydroxylase in man and animals will continue. The genetic studies of the control of enzymes will be generalized to the other enzymes in the synthesis pathway for adrenaline, and the relevance of these genetic differences to behavior will be examined.

Honors and Awards: Dr. Axelrod has been invited to give the Mike Hogg Lecture, University of Texas; the Rennebohm Lecture, University of Missouri; the Solomon A. Berson Memorial Lecture, Mt. Sinai Hospital; and the Alfred Burger Lecture, University of Virginia. He also received an honorary L.L.D. from City College of New York, and acted as proctor for the Honors Course at the University of Dartmouth. Dr. Coyle was an invited participant in the Neuroscience Research Program at Boulder, Colorado. He has been invited to present papers at an international symposium on neuronal growth at Stockholm and at the Third International Symposium on Catecholamines at Strasbourg, France, in addition to giving seminars at the University of South Carolina, NHLI, and NICHD. Dr. Ciaranello has been asked to give seminars at Dartmouth, Stanford, and the Universities of Colorado and Chicago, and to participate in the International Symposium at Strasbourg.

Publications:

Axelrod, J.: Biochemical pharmacology of catecholamines and its clinical implications. (1971 Foster Eltring Bennett Memorial Lecture). Trans. Am. Neurol. Assoc. 96: 179-186, 1972.

Axelrod, J.: Catecholamine metabolism, cardiovascular drugs and hypertension. In: Proceedings of the International Symposium on Catecholamines, Milan, Italy, 1970, Minerva Medica, Torino, 1972.

Axelrod, J.: Biogenic amines and their impact in psychiatry. Seminars in Psychiat. 4: 199-210, 1972.

Axelrod, J.: Chapter 14. Phenylethanolamine N-methyltransferase. Chapter 15. Enzymes in the metabolism of catecholamines I: Catechol-O-methyltransferase. Chapter 16. Enzymes in the metabolism of catecholamines II: Monoamine oxidase. In: Methods in Investigative and Diagnostic Endogrinology, Berson S.A. (Ed.), Vol. I, North Holland Publishing Company, Amsterdam, 1972. pp 536-550.

Axelrod, J.: Dopamine-β-hydroxylase: regulation of its synthesis and release from nerve terminals. Pharmacol. Rev. 24: 233-243, 1972.

Axelrod, J.: Neural and hormonal control of catecholamine synthesis. In: Research Publications: Association for Research in Nervous and Mental Disease, Kopin, I.J. (Ed.), Vol. 50, Williams and Wilkins Company, Baltimore, 1972, pp. 229-240.

Axelrod, J. and Weinshilboum, R.: Catecholamines. New Engl. J. Med. 287: 237-242, 1972.

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Molinoff, P.B. and Axelrod, J.: Distribution and turnover of octopamine in tissues. J. Neurochem. 19: 157-163, 1972.

Molinoff, P.B., Brimijoin, S. and Axelrod, J.: Induction of dopamine-β-hydroxylase in rat hearts and sympathetic ganglia. J. Pharmacol. Exp. Ther. 182: 116-130, 1972.

Wooten, G.F., Eldridge, R., Axelrod, J. and Stern, R.S.: Elevated plasma dopamine-β-hydroxylase activity in autosomal dominant torsion dystonia. New Engl. J. Med. 288: 284-287, 1973.

Wooten, G.F. and Cardon, P.: Plasma dopamine-β-hydroxylase activity. Arch. Neurol. 28: 103-106, 1973.

Coyle, J.T.: Tyrosine hydroxylase in rat brain: cofactor requirements, regional and subcellular distribution. Biochem. Pharmacol. 21: 1935-1944, 1972.

Coyle, J.T. and Wooten, G.F.: Rapid axonal transport of tyrosine hydroxylase and dopamine-β-hydroxylase. Brain Res. 44: 701-704, 1972.

Serial No. M-CS-Ph-6
1. Laboratory of Clinical Science
2. Section on Pharmacology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Tryptamine and other biogenic amines and psychoactive drugs

Previous Serial Number: Same

Principal Investigators: Julius Axelrod, Michael Brownstein
Juan Saavedra (Guest Worker) and David Kreuz

Man Years:		
	Total	3.0
	Professional	3.0
	Other	0

Project Description:

Objectives: To develop methods for the measurement of psychoactive drugs and normally occurring amines. To examine the formation and metabolism of tryptamine, phenylethanolamine, octopamine, and phenylethylamine.

Methods Employed: Biochemical, pharmacological, enzymatic and radiochemical techniques.

Major Findings: Enzymatic methods have been developed to measure picogram amounts of serotonin, phenylethanolamine, phenylethylamine and amphetamine. Phenylethanolamine and phenylethylamine were found to be normally occurring in brain and other tissues. Tryptamine and phenylethanolamine were found to be formed by the decarboxylation of tryptophan and phenylalanine, respectively. Inhibition of l-aromatic acid decarboxylase or monoamine oxidase raise the levels of brain tryptamine and phenylethanolamine and phenylethylamine. An enzyme that methylates tryptamine was found to be present in human platelets. This enzyme is elevated in platelets of schizophrenic patients.

Significance to Biomedical Research and the Program of the Institute: Blood levels of amphetamine can now be measured after taking small amounts of the drug.

Proposed Course of Project: The role of octopamine and other non-catechol biogenic amines will be examined. Serotonin and other biogenic amines will be examined in separate nuclei of the hypothalamus.

Honors and Awards: None

Publications:

Brandau, K. and Axelrod, J.: The biosynthesis of octopamine. Naunyn-Schmiedeberg's Arch. Pharmakol. 273: 123-133, 1972.

Cohn, C.K., Vesell, E.S. and Axelrod, J.: Studies of a methionine-activating enzyme. Biochem. Pharmacol. 21: 803-809, 1972.

Saavedra, J.M. and Axelrod, J.: A specific and sensitive enzymatic assay for tryptamine in tissues. J. Pharmacol. Exp. Ther. 182: 363-369, 1972.

Kreuz, D. and Axelrod, J.: Delta-9-tetrahydrocannabinol: localization in body fat. Science 179: 391-392, 1973.

Saavedra, J.M. and Axelrod, J.: Demonstration and distribution of phenylethanolamine in brain and other tissues. Proc. Nat. Acad. Sci. 70: 769-772, 1973.

Serial No. M-CS-Ph-7
1. Laboratory of Clinical Science
2. Section on Pharmacology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical and pharmacological studies on the pineal gland

Previous Serial Number: Same

Principal Investigators: Takeo Deguchi (Visiting Scientist), Michael Brownstein and Ronald Holz

Other Investigators: Julius Axelrod

Man Years:	Total	4.5
	Professional	2.5
	Other	2.0

Project Description:

Objectives: To examine how the sympathetic nervous system controls the pineal gland.

Methods Employed: Biochemical, pharmacological and radioactive tracer techniques.

Major Findings: The β-adrenergic receptor was found to control the circadian rhythms in pineal serotonin, N-acetylserotonin and N-acetyltransferase. A circadian rhythm in turnover of noradrenaline in pineal sympathetic nerves was found which would indicate that noradrenaline was released from nerves only during the nighttime. Bilateral lesions in the superchiasmatic nucleus in the brain abolishes the pineal rhythms. Depletion of noradrenaline by drugs causes pineal supersensitivity, while repeated administration of catecholamines results in subsensitivity. N-acetyltransferase in the pineal is rapidly degraded when the β-receptor is not occupied by a catecholamine.

Significance to Biomedical Research and the Program of the Institute: Circadian rhythms are important to action of drugs and hormones in the onset of disease. Our work explains how certain circadian rhythms are controlled.

Proposed Course of Project: Further studies on the mechanism of super- and subsensitivity will be explored. The role of the β-adrenergic receptors in protein synthesis will be examined.

Honors and Awards: Dr. Axelrod has been invited to give the annual Sigma Xi lecture. He has also been asked to serve on the editorial board of LIFE SCIENCES. Dr. Deguchi has been invited to lecture on the pineal at M.I.T.

Publications:

Deguchi, T. and Axelrod, J.: Control of circadian change of serotonin N-acetyltransferase activity in the pineal organ by the β-adrenergic receptor. Proc. Nat. Acad. Sci. 69: 2547-2550, 1972.

Deguchi, T. and Axelrod, J.: Induction and superinduction of serotonin N-acetyltransferase by adrenergic drugs and denervation in the rat pineal. Proc. Nat. Acad. Sci. 69: 2208-2211, 1972.

Deguchi, T. and Axelrod, J.: Sensitive assay for serotonin N-acetyltransferase activity in rat pineal. Anal. Biochem. 50: 174-179, 1972.

Serial No. M-CS-ET-1
1. Laboratory of Clinical Science
2. Section on Experimental Therapeutics
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemistry, Pharmacology and Physiology of Monoamines and Other Central Neurotransmitters

Previous Serial Number: Same

Principal Investigator: Thomas N. Chase

Other Investigators: T. Stephen Balch, Jacob A. Brody, Hinrich Cramer, Joseph Fermaglich, George Glaubiger, Edna K. Gordon, David A. Jacobowitz, Irwin J. Kopin, Morris A. Lipton, Raymond J. Matta, Lorenz K.Y. Ng (Guest Worker), E. Williams Pelton (Guest Worker), Roger Weir (Guest Worker) and Anne C. Woods (Guest Worker)

Cooperating Units: Section on Medicine and Units on Clinical Biochemistry and Histopharmacology, Laboratory of Clinical Science, NIMH; Epidemiology Branch, NINDS; Department of Neurology, Georgetown University School of Medicine; Department of Psychiatry, North Carolina School of Medicine

Man Years:		
	Total	5.0
	Professional	3.0
	Other	2.0

Project Description:

Objectives:

1. To study the transport, metabolism, storage, release and postsynaptic interactions of monoamines and other putative central neurotransmitters.

2. To examine the functional role of monoamines and other suspected neurohumoral agents in the central nervous system of normal individuals and in patients manifesting neuropsychiatric disease.

3. To study the mechanism of action and therapeutic efficacy of drugs which modify brain function.

Methods Employed:

1. Preclinical studies utilize a) slices or homogenates of brain, peripheral nerve and other tissues which are incubated with radioactively

labeled amines and related compounds, then exposed to drugs or electrical stimulation; b) surgically prepared laboratory animals in which the concentration of labeled and endogenous compounds in brain, cerebrospinal fluid, serum and urine are determined under various experimental conditions following the systemic or intracranial injection of labeled amines and other substances.

2. Clinical studies are conducted in patients with neuropsychiatric disease and in normal volunteers. Under resting conditions and during the administration of drugs or physical procedures believed to influence central synaptic mechanisms, neurologic, psychiatric and psychologic parameters of brain function are correlated with the results of a) chemical assay of relevant compounds in various body fluids and tissues, b) radioisotope tracer procedures and c) electrical measurement of spontaneous and evoked cortical potentials.

Major Findings: Since the satisfactory demonstration of neuronal histofluorescent is dependent upon the freeze-drying of tissues soon after death, it has not, heretofore, been practical to apply this method to human post mortem specimens. Work conducted with Drs. T.S. Balch and D.A. Jacobowitz now indicates that incubation in an oxygenated, physiologic medium can restore neuronal fluorescence to dopamine-containing terminals in rat cerebral tissues maintained in situ for as long as 8 hours after death. Neither the addition of α-methylparatyrosine (an inhibitor of dopamine synthesis), L-tyrosine (the precursor of this amine), nor benztropine (an inhibitor of dopamine uptake) to the medium altered the histofluorescence of incubated tissues. Preliminary applications of this technique to human autopsy material suggest that it may now be possible to study the distribution of monoaminergic systems in man's brain and search for possible abnormalities in patients with such diseases as parkinsonism and schizophrenia.

Although abnormalities in monoamine-containing neuronal systems have been implicated in the pathogenesis of disorders of affective state and extrapyramidal function, the role of parallel neuronal pathways, especially those in which acetylcholine may serve as a neurotransmitter, have yet to be carefully explored. An isotopic tracer technique has been developed with Drs. G. Glaubiger and R. Matta which enables in vivo studies of central acetylcholine metabolism. Pharmacologic agents such as 5-hydroxtryptophan and parachlorophenylalanine, believed to act relatively specifically on serotonergic function, were found to have reciprocal effects on acetylcholine turnover. Lithium, which has been thought to ameliorate bipolar affective states by influencing noradrengic transmission, substantially inhibited choline uptake in central nervous tissues and diminished acetylcholine synthesis. Reserpine, whose pharmacologic effects have generally been attributed to central monoamine depletion, significantly accelerated the formation of this suspected neurotransmitter.

A study in cats perfused through the spinal subarachnoid space with radioactively labeled 5-hydroxyindoleacetic acid conducted with Drs. R. Weir and I.J. Kopin indicates that as much as 50% of the lumbar CSF content of this serotonin metabolite derives from cerebral rather than spinal cord metabolism. These results are compatible with recent clinical studies in suggesting that lumbar spinal fluid levels of 5-hydroxyindoleacetic acid can be used as an index of brain as well as spinal cord serotonin metabolism.

In collaboration with Dr. E.W. Pelton the ability of the developing rat brain to take up and release monoamines has been studied. Marked regional differences in the functional capacity of dopamine, norepinephrine and serotonin-containing neuronal systems have been observed at various stages of development. Attempts to isolate endogenously synthesized neurotoxins which might be accumulated by dopamine-containing nerve cells are now in progress. Of particular interest is the possibility that the mammalian brain synthesizes 6-hydroxydopamine whose ability to cause degeneration of central dopaminergic neurons is now well established.

Attempts to study central norepinephrine metabolism through assay of 3-methoxy-4-hydroxyphenylglycol (MHPG) in spinal fluid have continued in collaboration with E.K. Gordon and L.K.Y. Ng. Both α-methylparatyrosine, an inhibitor of tyrosine hydroxylase, and fusaric acid, an inhibitor of dopamine hydroxylase, were found to significantly diminish MHPG values. On the other hand, doses of L-dopa or probenecid, sufficient to substantially elevate CSF levels of the dopamine metabolite, homovanillic acid, failed to alter the spinal fluid content in MHPG. CSF concentrations of MHPG in patients with Parkinson's disease or with other central nervous system disorders studied did not differ significantly from control levels. Studies of spinal fluid levels of vanillyl mandelic acid (VMA), another important metabolite of norepinephrine, are now in progress.

A study of adenosine-3',5' monophosphate (cAMP) levels in the lumbar spinal fluid of 41 patients with peripheral or central nervous system disease conducted with Drs. H. Cramer and L.K.Y. Ng has now been completed. cAMP levels appeared unrelated to the age or sex of the patients or to the time of day that the samples were obtained. Levels in parkinsonian patients did not differ significantly from those with familial or acquired dystonia, spinal cerebellar disorders or those of control subjects. Neither the acute nor chronic administration of L-dopa altered cAMP concentrations. In contrast, probenecid caused a substantial rise in levels of this nucleotide. The latter results indicate that cAMP efflux from the spinal fluid compartment may be mediated by a probenecid sensitive transport system and the use of probenecid may provide a means for studying drug or disease effects on the central turnover of cAMP in man.

The probenecid loading technique continues to provide important new information relevant to the dynamic state of cerebral monoamines in man. Haloperidol, which is believed to block dopamine receptors, substantially increased central dopamine turnover as estimated by the probenecid technique while ET 495, a suspected dopamine receptor agonist, exerted the opposite effect. These observations indicate that a causal relation may exist in man between the activity of postsynaptic dopaminergic receptors and dopamine turnover in the presynaptic neuron. They also suggest that putative dopamine receptor agonists and antagonists may provide a means for evaluating mechanisms which regulate dopamine synthesis and release in patients with central nervous system diseases. Initial results in patients with Parkinson's disease, indicate that haloperidol produces little or no rise in dopamine turnover, presumably owing to the degeneration of dopaminergic neurons which attends this disorder. In Huntington's chorea patients, however, there was a marked increase in the probenecid-induced accumulation in homovanillic acid during haloperidol treatment. The diminished dopamine turnover in untreated patients with this disorder may thus reflect a functional inhibition of dopaminergic neurons secondary to the degeneration of striatal cells which receive their axonal terminals.

Studies of L-dopa alone or in combination with a peripheral decarboxylase inhibitor continue in an attempt to evaluate the long-term clinical effects of these drugs and more precisely elucidate their mechanisms of action. In a collaborative study on Guam with Drs. J.A. Brody and E.M. Holden, L-dopa-induced dyskinesias were found to occur in over 50% of patients with parkinsonism-dementia but in none of those with amyotrophic lateral sclerosis who were matched for dose and duration of L-dopa exposure. In parkinsonism-dementia patients no correlation was found between pretreatment parkinsonian severity or the degree of response to L-dopa and the appearance of dyskinesias. It would thus appear that lesions of the type which produce parkinsonism increase the susceptibility to L-dopa-induced dyskinesias but that denervation supersensitivity alone can not account for this phenomena.

The role of dopamine release from vescicular stores in mediating the clinical effects of L-dopa has been studied in parkinsonian patients. Individuals receiving a stable dose of L-dopa and were given increasing doses of tetrabenazine, which is known to block monoamine uptake into nerve terminal granules. To date no significant changes in the antiparkinsonian efficacy or toxicity of L-dopa have been observed. In related experiments the co-administration of drugs which inhibit monoamine uptake into specific monoaminergic systems have been studied in order to provide insight into the relative contribution of these neurons to the clinical effects of L-dopa. Imipramine (which selectively interferes with the transport of monoamines into serotonergic neurons) yptaline (which mainly inhibits monoamine uptake into noradrenergic neurons) were added to the therapeutic regimen of patients receiving L-dopa. Little, if any, change in the antiparkinsonian efficacy or toxicity of L-dopa was observed. These results suggest that serotonergic or noradrenergic neurons may not play a critical role in mediating either the therapeutic or toxic effects of L-dopa.

Clinical observations indicate that the antiparkinsonian response to L-dopa becomes increasingly brittle as the duration of therapy increases. Evidence that this "on-off" response to orally administered L-dopa largely reflects variations in circulating levels of the amino acid rather than to drug-induced changes in central dopaminergic mechanisms derive from ongoing studies conducted by Drs. A. Woods and G. Glaubiger. For each of the patients studied thus far the maintenance of a constant plasma L-dopa level, by means of a constant intravenous infusion of the amino acid, produced a stable clinical response. These observations have now prompted clinical trials of a sustained release preparation of L-dopa.

Preliminary results of a double-blind, cross-over study conducted in parkinsonian patients with Dr. J. Fermaglich indicate that at appropriate dose levels, methyldopa, a marketed antihypertensive agent, is as effective a potentiator of L-dopa as the experimental compound, α-methyldopahydrazine (MK 486). Moreover, the ability of either agent to overcome L-dopa-induced adverse effects were indistinguishable. Although these results may be attributable to the peripheral effects of methyldopa and MK 486, the ability of methyldopa to enter the central nervous system requires consideration of other mechanisms by which decarboxylase inhibitors potentiate the antiparkinsonian action of L-dopa.

Attempts to modify central nervous system function with newly developed pharmacologic agents continue. The antiparkinsonian efficacy of ET 495, a suspected dopamine receptor agonist, has now been studied in 6 patients. Despite the marked changes in central dopamine metabolism already described, only mild symptomatic relief occurred. Moreover, several patients developed abnormal involuntary movements suggesting that mechanisms mediating the ability of L-dopa to ameliorate parkinsonian signs may differ from those relating to the induction of dyskinesias. On the basis of recent observations that thyrotropin releasing hormone (TRH) potentiates the motor effects of L-dopa in rodents, clinical studies of the ability of this hypothalamic tripeptide to enhance the antiparkinsonian efficacy of L-dopa have now begun in collaboration with Dr. M.A. Lipton. Attempts to elucidate the relationship of GABA-containing neuronal systems to psychomotor function in man are now in progress using BA-34,647, a centrally active p-chlorophenyl derivative of this suspected central neuromediator.

Significance to Biomedical Research and the Program of the Institute: The Section seeks to develop by rational means improved pharmacologic treatments for neuropsychiatric disease. Most experimental efforts continue to focus on neurohumoral mechanisms, since substances participating in this process appear to act as sensitive indicators of central nervous system function and constitute the site of action of many drugs which affect psychomotor behavior. Alterations in central monoamines found in relation to several extrapyramidal and behavioral disorders have lead to the discovery of drugs which reverse both the biochemical and clinical defects. This approach to the development of new pharmacologic therapy may be applicable to other disorders of brain function.

Proposed Course of Project: Studies of monoamines in relation to naturally occurring and drug-induced neuropsychiatric disease will continue. Attempts to improve our ability to evaluate central monoaminergic mechanisms in man by means of an intravenous probenecid test combined with isotopic tracer infusions will be undertaken. Increased attention will also be accorded current efforts to develop techniques for evaluating other suspected central neurotransmitters including γ-aminobutyric acid and the prostaglandins. The role of monoaminergic systems in mediating pain perception will also be examined.

Honors and Awards: During the past year Dr. Chase was elected to membership in the American Neurologic Association and the American Academy of Neurology. He was invited to serve as a member of the Neurology Advisory Committee and as a consultant to the Neuropharmacology Advisory Committee of the Food and Drug Administration, and to the Advisory Editorial Board of Neuropharmacology. Invitational lectures or seminars were given at the Fourth International Symposium on Parkinson's Disease, Zurich, Switzerland, September 20-25, 1972; The Coatesville Veterans Administration Hospital and the Chester County Medical Society, October 18, 1972; Food and Drug Administration, October 26, 1972; International Symposium on Trivastal, Monastir, Tunisia, November 27-December 1, 1972; CCHD Workshop on Huntington's Chorea, Los Angeles, California, January 19-21, 1973; California Institute of Technology, Pasadena, California, March 27, 1973; Princeton Research Conference on Parkinson's Disease, April 11-13, 1973; Veterans Administration Hospital Symposium on Huntington's Chorea, Knoxville, Iowa, May 1, 1973; George Washington University School of Medicine, Washington, D.C., May 7, 1973; NIH Clinical Staff Conference on Dyskinesias of the Mouth, Pharnynx and Larynx, May 10, 1973; International Conference on Catecholamines and Their Enzymes in the Neuropathology of Schizophrenia, Strasbourg, France, May 19-20, 1973; III International Catecholamine Symposium, Strasbourg, France, May 20-25, 1973; International Symposium on 5-Hydroxytryptamine and Other Indolealkylamines in Brain, Cagliari, Italy, May 27-June 2, 1973. Dr. Chase also appeared on a televised discussion of Parkinson's Disease on Eurovision I, December 2, 1972 and served as an invited discussant of papers entitled "Saluatory Effects of L-dopa on Dystonia Musculorum Deformans," "Hypoxia Alters Cerebral Monoamine Metabolism in the Rat," "Abnormalities in H^3-tyrosine in Parkinson's Disease," and "Brain Monoamine Turnover of Children: Preliminary Results in Epilepsy, Minimal Brain Dysfunction and Movement Disorders" at the 25th Annual Meeting of the American Academy of Neurology, Boston, April 23-28, 1973.

Publications:

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Ng, L.K.Y., Chase, T.N., Colburn, R.W. and Kopin, I.J.: L-dopa in parkinsonism: A possible mechanism of action. Neurology 22:688-696, 1972.

Cramer, H., Ng, L.K.Y. and Chase, T.N.: Effect of probenecid on cyclic AMP levels in human spinal fluid. J. Neurochem. 19:1601-1602, 1972.

Chase, T.N.: Drug-induced extrapyramidal disorders. Res. Publ. Assn. Nerv. Ment. Dis. 50:448-471, 1972.

Lott, I.T., Murphy, D.L. and Chase, T.N.: Down's syndrome: Central monoamine turnover in patients with diminished platelet serotonin. Neurology 22: 967-972, 1972.

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Ng, L.K.Y., Chase, T.N., Colburn, R.W. and Kopin, I.J.: Release of H^3-dopamine by 5-hydroxytryptophan. Brain Res. 45:499-505, 1972.

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Mendell, J.R., Murphy, D.L., Engel, W.K., Chase, T.N. and Gordon, E.K.: Catecholamines and indoleamines in patients with Duchenne muscular dystrophy. Arch. Neurol. 27:518-520, 1972.

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Chase, T.N.: Biochemical and pharmacologic studies of monoamines in Huntington's chorea. In *Huntington's Chorea,* Barbeau, A., Chase, T.N. and Paulson, G.W. (eds.), Raven Press, New York, 1973.

Eldridge, R., O'Meara, K., Chase, T.N. and Donnelly, E.F.: Offspring of consanguineous parents with Huntington's disease. In *Huntington's Chorea,* Barbeau, A., Chase, T.N. and Paulson, G.W. (eds.), Raven Press, New York, 1973.

Balch T.S., Chase, T.N. and Jacobowitz, D.: Dopamine histofluorescence: Restoration in mammalian brain tissues prepared several hours post mortem. Brain Res. 52:419-423, 1973.

Chase, T.N. and Murphy, D.L.: Serotonin and central nervous system function. Ann. Rev. Pharmacol. In press.

Weir, R.L., Chase, T.N., Ng, L.K.Y. and Kopin, I.J.: 5-hydroxyindoleacetic acid in spinal fluid: Relative contribution from brain and spinal cord. Brain Res. In press.

Serial No. M-P-C-(C)-12
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of heredity and environment in schizophrenia.

Previous Serial Number: Same.

Principal Investigator: David Rosenthal

Other Investigators: Paul H. Wender, Seymour S. Kety (Harvard), Shmuel Nagler (Israel), Fini Schulsinger (Denmark).

Cooperating Units: None.

Man Years:

Total:	7.0
Professional:	3.0
Others:	4.0

Project Description:

Objectives: To understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

Methods Employed: 1. A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. 2. An intensive multidisciplinary study of a family with one-egg quadruplet daughters concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. 3. Studies of adoptees and their biological and adoptive families. 4. A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

Major Findings: 1. The analysis of the literature has been reported in a series of papers and in the study of the quadruplets. A textbook which organizes and explicates the literature on the genetics of behavioral disorders, and a modified version of this book have been published. 2. Several papers on our adoption studies are now published. The studies indicate that hereditary factors contribute to the development of schizophrenia, and to other disorders that we include in a group called the schizophrenia spectrum. Several articles are in preparation and eventually a monograph will be written on the adoption studies. Data analysis is continuing. 3. One hundred subjects in the Israel study have been examined, and the research findings are currently being analyzed and evaluated. Preliminary findings indicate that offspring of schizophrenics have more neuropathological signs in childhood than do controls. Two subjects have had a breakdown and one is on the verge of breakdown. All three have a schizophrenic parent.

Significance to Bio-medical Research and the Program of the Institute: We have resolved a chronic, critical problem in psychiatry by demonstrating beyond any reasonable doubt that genetic factors are importantly involved in the etiology of schizophrenia and related mental disorders. Moreover, the evidence thus far suggests that the mode of genetic transmission is polygenic or a dominant single gene with polygenic modifiers.

Proposed Course of Project: We are examining additional subjects in Denmark with the following goals in mind: 1. to try to discriminate gene carriers and non-gene carriers with respect to personality and test variables; 2. to compare the fate of gene-carriers who are reared in the parental home as compared to those reared in adoptive homes; 3. to compare Ss who have a psychotic biological parent but who are reared by nonpsychotics with Ss whose biological parents are not psychotic but who are reared by a psychotic adoptive parent; 4. to find the incidence of schizophrenia spectrum disorder in a random sample of the Danish population. In Israel, our goal is to prepare a volume on our findings and their theoretical implications. During this year we will carry out reexaminations of this unique sample of subjects.

Honors and Awards: None.

Publications:

Manne, S. H., and Rosenthal, D.: IQ and age of first commitment of dangerous offenders. J. Am. Assoc. Correctional Psychologists 4: 220-229, 1971.

Rosenthal, D.: Review of E. Slater, and V. A. Cowie, The genetics of mental disorders. J. Nerv. Ment. Dis., 155: 296-297, 1972.

Rosenthal, D.: Hereditary nature of schizophrenia. In Kety, S. S., and Matthysse, S. (eds.): Neurosci. Res. Progr. Bull. Brookline, Mass., 1972, 10: 397-403.

Rosenthal, D.: Reivew of I. I. Gottesman, and J. Shields, Schizophrenia and Genetics. Science, 179: 1117-1118, 1973.

Wender, P. H., Rosenthal, D., Kety, S. S., Schulsinger, F., and Welner, J.: Social class and psychopathology in adoptees: an experimental method for separating the roles of genetic and experiential factors. Arch. Gen. Psychiatry 28: 318-325, 1973.

Serial No. M-P-C(C) - #15

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Reaction time in schizophrenia.

Previous Serial Number: Same

Principal Investigator: Theodore P. Zahn

Other Investigator: Paul Fedio

Cooperating Unit: Surgical Neurology Branch, NINDS

Man Years:
Total: .2
Professional: .1
Other: .1

Project Description:

Objectives: To study deficits in attention in schizophrenia, particularly as manifested in difficulties in adopting and maintaining preparatory sets. To study the specificity of such deficits to schizophrenia. To study possible organic and genetic determinants of such deficits. To determine the relation between such deficits and the severity of schizophrenic symptomatology.

Methods Employed: (A) A reaction time (RT) technique which puts severe demands on attention and which discriminates well between schizophrenic and non-schizophrenic subjects is being given to patients before and after unilateral temporal lobectomy for the relief of psychomotor epilepsy. This procedure and a choice technique which compared RT to monaural stimulation in each ear and responses with the same or opposite hand have also been used on postoperative temporal lobe followup cases and normal controls.

(B) Acute schizophrenic and non-schizophrenic patients are being tested with these procedures soon after admission, on discharge from the Clinical Center after 3-4 months and on a followup 6 months to 1 year later. The results will be compared with intensive symptom ratings.

Major Findings: We continue to find that about half of the temporal lobe followup cases give essentially normal performance despite some apparent personality disturbance. Most patients tested 10 - 15 days postoperatively have shown a decline from their preoperative performance level, suggesting a possible recovery of function over time. The followup cases are also slower in choice RT but further analysis of the data is needed to determine if this slowing is disproportionate to the simple RT level.

Significance to Biomedical Research and the Program of the Institute: These studies are designed in part to explore the possibility of a biological basis for schizophrenia. Since patients with lesions in the temporal lobe frequently exhibit a schizophrenic-like personality picture and since patients with other types of brain lesions have been found to exhibit reaction time deficits (but of different types than those shown by schizophrenics) the reaction time performance of these patients might indicate if a temporal lobe disturbance is possibly involved in schizophrenia. The reaction time studies with schizophrenics add to our knowledge of the specific psychological processes involved in the disorders of set and attention that seem to be important aspects of the schizophrenia process.

Proposed Course of Project: Further collection of data on both current and followup temporal lobe cases. When enough data have been collected the effects of the side of the lesion and correlations with the amount of tissue removed can be determined. Further data collection on schizophrenic and non-schizophrenic patients.

Honors and Awards: None.

Publications: None.

Serial No. M-P-C-(C) - #17
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS - HSMHA - NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychophysiological Responsivity in Schizophrenia.

Previous Serial Number: Same.

Principal Investigator: Theodore P. Zahn

Other Investigators: David Rosenthal, William Pollin, Loren Mosher

Cooperating Units: Section on Twin and Sibling Studies, APB, NIMH
Section on Technical Development, MHIRP
Section on Psychiatric Assessment, APB, NIMH

Man Years:

Total:	.5
Professional:	.2
Other:	.3

Project Description:

Objectives: The general purpose of these studies is to investigate schizophrenic-normal differences in the relative autonomic responsivity to various stimuli, the relationship of autonomic activity to adequacy of performance and to psychiatric condition, and possible genetic determinants.

Methods Employed:

1. Peripheral measures of autonomic functioning, namely GSR, heart rate, finger pulse volume, respiration and skin temperature are recorded during several sessions in which stimuli are presented and tasks are performed which vary in the demands placed on the subjects: no stimuli, simple auditory stimuli with no response required, the same with a "casual" response required, reaction time, word association, mental arithmetic, and cold presser. Specific and non-specific response frequencies and amplitudes, baseline values and baseline changes are measured. These procedures have been used with identical twins discordant or concordant for schizophrenia and their parents and with the adoptive and biological parents of schizophrenic patients in order to see if whatever genetic influence there is in schizophrenia is manifested in autonomic functioning.

Currently, acute schizophrenic and non-schizophrenic patients are being tested on a shorter version of the above procedures soon after admission, at discharge from the Clinical Center (3-4 months later) and at a 6 month to 1 year followup.

Methods have been developed in our laboratory to analyze skin conductance, heart rate, skin temperature and finger pulse volume data automatically from taped analog records by means of the SEL 810 B computer.

2. A large scale exploratory correlational analysis using both univariate and multivariate (canonical) correlations of the psychophysiological variables with biochemical and psychiatric data collected by other investigators is being carried out to determine the interrelationships between these major classes of variables.

3. Analyses of the genetic determinants of psychophysiological variables on the data from the twins is being carried out by a method of intra-class correlation which attempts to control for any consistent influence of psychopathology on the dependent variable.

Major Findings: 1. Previously we had found that the differences between the schizophrenic and non-schizophrenic members of the twin pairs were similar to the differences between unrelated schizophrenic and normal subjects in that: (a) despite equal autonomic responsivity to meaningless stimuli, the schizophrenics were significantly hyporeactive to more meaningful stimuli; and (b) the schizophrenics showed higher arousal levels under nondemanding conditions but the controls showed a greater increase in arousal under the mild stress of task performance. The latter group, in fact, showed some tendency to be hyperreactive to meaningful and/or stressful stimuli compared to normal subjects who do not have a familial history of schizophrenia.

2. Preliminary impressions of the data currently being collected on acute schizophrenics suggest that when the patients are exhibiting acute schizophrenic symptomatology, they show the hyporeactivity to meaningful stimuli as described above, but become markedly more reactive, perhaps hyperreactive, during periods of remission from the schizophrenic symptoms.

3. Preliminary results from the correlational analyses show unexpected but consistent negative correlations between electrodermal and heart rate indices of arousal and urinary measures of the precursors and metabolites of epinephrine and norepinephrine (dopamine, metanephrine, and normetanephrine). Correlations among the autonomic performance and behavioral variables suggest that schizophrenics with high resting (nonspecific) arousal levels have poorer performance and more severe symptomatology than those with lower arousal levels, while the relationships of task produced increments in arousal have just the opposite relationships with the behavioral variables. Similar, but much less marked relationships are found in controls.

4. Preliminary analyses of the genetic determination of psychophysiological variables have shown that overall autonomic arousal levels, variability and responsivity seem to have a significant genetic-familial

component, while performance measures and the autonomic response to demand do not show appreciable genetic influence.

5. A review of the literature on the relationships of autonomic phenomena to behavior in schizophrenia has revealed much support for the implication from our research that there is no generalized deficit in autonomic reactivity in schizophrenia, but that such deficits occur specifically to meaningful or demanding stimuli such as those involved in task performance. It is also apparent from the literature that a simple inverted U relation between autonomic arousal and performance is not well confirmed, and that what we have called specific and non-specific arousal might have different relationships to behavior in schizophrenia.

Significance to Biomedical Research and the Program of the Institute: The role of autonomic arousal and responsivity in schizophrenic deficits in behavior remains obscure, but it is clear from our own research and that of other investigators that a low arousal level is not the critical factor as has sometimes been postulated. Our evidence suggests that a high arousal level that is non-task-related (non-specific arousal) and low task-related increments in arousal or the relationship between these may be related to such performance deficits. Further elucidation of the autonomic concomitants of symptoms and behavior deficits in schizophrenia may give us an increased understanding of the biological aspects of this condition and therefore may lead to more specific therapeutic procedures.

In view of the abundant evidence that genetic factors are of importance in the etiology of schizophrenia, it is reasonable to ask if a "schizophrenia genotype" is manifested in the autonomic nervous system. Some suggestions of autonomic hyperactivity in our co-twins indicate that if there is a genetically determined autonomic pattern that predisposes individuals to schizophrenia it is of a markedly different nature than that shown by individuals who are, in fact, schizophrenic. This conclusion receives some indirect support from our impression that subjects who have remitted from an acute schizophrenic episode seem to show a similar pattern of autonomic response as the non-schizophrenic co-twins.

Proposed Course of Project: 1. Further analysis of the data from the twins and their families will include (a) examining more autonomic variables such as vasomotor changes, and other aspects of electrodermal functioning based on new findings in the literature; (b) multivariate statistical analysis of group differences; and (c) partial correlations on selected subsets of the large set of variables from different investigators.

2. The development of techniques to manipulate general arousal levels and specific alerting systems independently. The use of shock to reinforce slow reaction times and in a non-contingent fashion may be a promising start on this problem.

3. More precise measurement of cardiac responsivity will be attempted by means of estimation techniques based on auto-regressive and

cross-regressive models of heart rate variation.

4. Continued data collection on acute subjects.

Honors and Awards: None.

Publications: Zahn, T. P. Psychophysiological concomitants of task performance in schizophrenics. In: Zubin, J., Sutton, S., and Keitzman, M. (Eds.) Experimental Approaches to Psychopathology. Academic Press. In press.

Serial No. M-P-C-(C) - 36
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS - HSMHA - NIMH
Individual Project Reports
July 1, 1972 through June 30, 1973

Project Title: Psychological Correlates of Cortical Evoked Responses.

Previous Serial Number: Same

Principal Investigator: Monte Buchsbaum

Other Investigators: Stephen G. Landau, Thomas Bittker, Dennis Murphy, Frederick Goodwin and Robert I. Henkin

Cooperating Units:

Adult Psychiatry Branch, NIMH
Laboratory of Clinical Science, NIMH
Clinical Endocrinology, NHLI

Man Years:

Total:	2.2
Professional:	1.2
Other:	1.0

Objectives: Exploration of relationships between perceptual response patterns, psychiatric clinical dimensions and neurophysiological measures; the development of improved techniques for collecting and analyzing neurophysiological data collected from intact human subjects; detailed study of the interaction between attention, cortical area and individual differences in neurophysiological response.

Methods Employed: Research in this project focuses on understanding the psychophysiology of perception and thus providing a background for studies of perceptual and cognitive disturbances in psychiatric patients. Its work is closely coordinated with clinical research in the section on Perceptual and Cognitive Studies, Adult Psychiatry Branch.

Central nervous system function has been difficult to study in psychiatric patients by either conventional neuroanatomic or implanted electrode techniques, as the former requires a dead patient (or an animal model of the disease) and the latter requires a hazardous surgical procedure. Obviously, neither method permits long-term study of psychiatric episodes. With the development of average evoked response techniques, which allow recovery of relatively specific CNS signals from scalp EEG, neurophysiological attempts to study psychiatric disorders received new impetus. The average evoked response (AER) is a particularly promising tool for studying perception in the psychiatric patient because unlike traditional psychophysical techniques, it does not depend upon the patient's report or overt performance.

Thus, the importance of motivation or motor activity is minimized and a clearer separation of perceptual (input) and performance (output) factors may be achieved.

Cortical average evoked responses (AER's) are patterns of electrical response to sensory input which are recorded from the electroencephalogram. Because evoked responses are usually of extremely small amplitude (less than 10 millionths of a volt), these patterns are completely lost in the apparently random fluction of the EEG. But we can overcome this obstacle by taking advantage of the fact that an evoked response is always related in time, or "time-locked," to a sensory stimulus, whereas the background EEG activity varies and bears no fixed relationship to the stimulus. By presenting the subject with a long series of stimuli, such as flashes of light, and summing the EEG for a brief time interval after each flash, an AER is produced.

The development of computer technology has been an essential step in the application of average evoked response measures to large scale patient testing. During the past year, our system for collection and processing of the evoked response data has been significantly expanded. A new capacity for precision control of auditory stimuli, and for electrical (somatosensory) stimuli has been developed. Furthermore, programs have been written to allow the subject's specific key press response to stimuli to be conveniently entered into the EEG data record. This will enable us to perform experiments linking the subject's subjective response to a stimulus to his electroencephalographic response. We have also developed programs for the easy transfer of data from the LINC computer through the SEL computer which enables us to use the data processing facilities of the SEL and the data collection facility of the LINC computer. These new programs have enabled investigators in other laboratories new to the evoked response field to begin using the SEL.

We have also continued to share our PDP-12 software and have sent our systems tapes to investigators at the University of Rochester, Duke University Medical Center, St. Elizabeth's Hospital and program write-ups to several other interested potential users in the United States, Canada and England.

Major Findings: Coping with sensory overload is perhaps the major perceptual problem of 20th century urban man; individuals differ widely both in their relative success in meeting the challenge and in their styles of coping. Evoked responses to loud, repetitious, meaningless and meaningful stimuli have been studied in subjects attending to or trying to ignore the stimuli. The early discovery that some subjects paradoxically showed actual decreases in AER amplitude with increasing stimulus intensity led to the development of a perceptual style concept known as "stimulus intensity control," and to a variety of clinical and more basic investigations.

1. Methodologic Issues. In comparisons of the EEG recorded from the occipital cortex (visual area) and the vertex (non-specific areas) we have found that both pupillary diameter and the area of the visual stimulus

have much larger effects over the visual cortex than at non-specific areas. In contrast, stimulus expectancy, randomization and spacing effects are more prominent at the vertex. This area specificity gives us further evidence that central rather than peripheral mechanisms are involved in the observed psychological effects.

2. Biochemical and Clinical Studies. These studies are reviewed in M-AP(C) - 21-2, Studies of Perceptual and Cognitive "Styles" in Psychiatric and Non-psychiatric Subjects.

3. Arousal and Attention. The effects of shifting attention toward or away from visual or auditory stimuli of varying intensities with a study using evoked responses in 24 normal human volunteers. Subjects were asked to attend to visual or auditory stimuli of four intensities (randomly presented) or to ignore the lights and tones and do mental arithmetic. For visual stimuli, attentional effects were the largest at low intensities, whereas for auditory stimuli equal effects were shown across all intensities. Similar individual rates of increased AER amplitude with increasing intensity were observed for both visual and auditory stimuli when attentional conditions were controlled. These results suggest that some general intensity processing response operating across sensory modalities is reflected in the AER.

4. Twin Studies. Data on the average evoked response is reported in project M-AP(C) - 21-4. Average evoked responses, perceptual tasks, autonomic psychophysiology, blood type and interview material have been collected on 60 normal twin pairs. Detailed analysis of the evoked response results has revealed high heritability especially in positive rather than negative evoked response components, and higher heritability for functional relationships, such as the amplitude intensity function, than for pure amplitude or latency measures of a single response. The amplitude-intensity slope was calculated using the time coordinant of the evoked response to the four intensities of light at 4 msec intervals. This yielded the rate of change in evoked response amplitude at each point in time following the stimulus. The amplitude-intensity slope calculated in this fashion reached its lowest point, a negative value at almost exactly 100 msec and its highest point at almost exactly 200 msec; this minima and maxima correspond almost exactly with the mean latencies observed for the two positive components of the evoked response. Heritability of the slope measure was calculated based on our samples of 30 MZ and 30 DZ twin pairs. Heritability peaked precisely at the time of the two positive components, reaching levels of approximately 0.7. In general the slope measure had far higher heritability than any raw amplitude measure. This tends to suggest that we are indeed looking at the heritability of a functional neurophysiological property of the nervous system rather than the heritability of perhaps arbitrary anatomical characteristics. We have also roentgenographically examined the relationship between skull thickness and scalp thickness and measures of the evoked response; no relationships were found.

5. Sex Differences The relationships between personality measures and AER measures of perceptual differentiation and stimulus intensity control were studied in two groups of male and female subjects. Significantly different patterns of sex differences and of correlations between the two types of evoked response measures were found in personality and clinical indices of adjustment. Maladjusted individuals evidence AER and perceptual responses prototypic of the opposite sex. This is consistent with our investigations in patients with affective disorders; interactions between intensity, patient's sex, and diagnosis were prominent.

Visual and auditory evoked responses were studied in a separate large study in 166 normal males and females from 6 to 60 and in ten patients with chromatin negative gonadal dysgenesis. Females of all ages and patients with 45XO exhibited larger amplitude AER than men. Younger subjects exhibit larger AER amplitudes and greater increases in amplitude with increasing stimulus intensity; latencies were shorter and increased with increasing intensity, rather than decreasing as in older subjects. These findings suggest that levels of FSH, LH, or gonadal steroids cannot be the only determinants of sex differences observed. From the data on patients with 45XO it may be hypothesized that the absence of the Y chromosome may be associated with the female AER pattern.

Proposed Course of Project.

1. Clinical studies are summarized in project M-AP(C) - 21-2.

2. Twin studies. Data analysis on variables, eye movements, and the battery of other evoked response procedures is continuing (see M-AP(C) 21-4.

3. Habituation. Parallels between our human AER habituation results and Thompson's dual process theory of habituation (based on acute spinal cat data) were pursued in a large study utilizing several evoked response habituation paradigms at high and low intensities with subjects attending and distracted. These studies are complete and the data are being analyzed.

4. Attention. Lateralization in attentional effects is being studied using visual stimuli of four intensities presented to left and right hemispheres. Recent reports on unilateral electric shock therapy, brain lesions, carotid amytal and AER have suggested that lateralization or interhemispheric communication may be disturbed in psychiatric disorders; initial explorations in normal subjects have begun.

5. Sensitivity, Pain and Drug Addiction. Striking individual differences in response and self selection for medication are nowhere stronger effects than for addictive drugs. A variety of studies suggest that baseline AER (off-drug) differences exist between addict or psychiatric patient groups and normals and that these groups are "normalized" by

their drug dependance or greatest clinical efficacy. In a project with Dr. Arnold Ludwig at the University of Kentucky, the effects of alcohol on AER measures of augmenting-reducing will be investigated in alcoholics and normals under sensory overload and sensory deprivation. In collaboration with Dr. Tom Chase and Dr. Larry Ng, we will study AER responses to painful and nonpainful electrical stimuli. Parametric studies of intensity judgments, habituation, pain and channel capacity for information overload will be started in preparation for clinical studies.

Honors and Awards: None.

Publications:

Landau, S. G., and Buchsbaum, M. Average evoked response and muscle tension. Physiol. Psychol. 1 (1): 56-60, 1973.

Buchsbaum, M., King, C. and Henkin, R. I. Average evoked responses and psychophysical performance in patients with pseudohypoparathyroidism. J. Neurol. Neurosurg. Psychol. 35 (2): 270-276, 1972

Buchsbaum, M. and Coppola, R. Computer use in bioelectric data collection and analysis. In: Bioelectric Recording Techniques (Eds) Thompson, R. and Patterson, F., Academic Press, in press.

Schechter, G. and Buchsbaum, M. The effects of attention, stimulus intensity and individual differences on the average evoked response. Psychophysiology, in press.

Serial No. M-P-C-(C)-39
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of heredity and environmental factors in schizophrenia.

Previous Serial Number: Same

Principal Investigator: Paul H. Wender

Other Investigators: David Rosenthal, John Rainer (New York)
Laurence Greenhill

Cooperating Units: Research Foundation for Mental Hygiene, Inc.,
722 West 168th Street, New York, N.Y. 10032

Man Years:

Total:	4.1
Professional:	3.7
Other:	.4

Project Description:

Objectives: To assess the role of environmental factors in the etiology of schizophrenia.

Methods Employed: The strategy involves the use of adopted subjects to separate the roles of genetic and experiential factors. Since the biological parents are not the parents who rear their children, the transmitters of genetic factors and social experience are separated. In this study we evaluate the psychiatric status and cognitive function of three groups of parents: those who have adopted children who subsequently became schizophrenic; those who reared their own schizophrenic children; and those who reared their own organically impaired offspring.

Psychiatric pathology is assessed by means of structured interviews administered by interviewers who are blind as to the status of the parents interviewed. Cognitive function is assessed by means of a battery of psychological tests.

The design permits a test of three hypotheses: (1) if psychopathology among the parents of schizophrenics is a cause of the disturbance in the offspring it should be present in both the adopting and biological parents of schizophrenics; (2) if psychopathology in the parents of schizophrenics is largely a manifestation of a genetically transmitted disorder, it should be present in the biological and not the adopting parents of schizophrenics;

(3) if psychopathology among the parents of schizophrenics is largely reactive to pathology in the child, it should be present equally in the parents of schizophrenics and of organically impaired children.

The present study, which replicates an earlier study of the adopted parents of schizophrenics, has three methodological improvements: (1) it employs systematic sampling; (2) it employs raters who are kept blind as to the status of the parents interviewed; (3) it employs a comparison group of parents of organically impaired children (controlling for the effect of the child on the parent).

Major Findings: Case findings and evaluation have been completed. Fifty-eight couples (of the sixty proposed) have been interviewed and tested. Q-sorts of psychiatric pathology have been performed. This analysis has shown that the adopting parents of Szs and the natural parents of retardates show equivalent degrees of psychopathology and that both groups are very significantly less disturbed than the natural parents of Szs. Similarly, the word association test has shown the natural parents of Szs to be significantly more abberant than the other two parent groups. The results thus confirm those of an earlier pilot study.

Significance to Bio-medical Research and the Program of the Institute : We are attempting to validate our earlier finding which indicated that parental psychopathology did not seem strongly related to the etiology of schizophrenia.

Proposed Course of Project: Transcription of the interview and test material should be completed by July 1973. Data analysis will begin at that time and should be complete -- except for the Rorschach evaluations -- by the end of 1973.

Honors and Awards: None.

Publications: None.

Serial No. M-P-C-(C)-40
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Changes in neurological and psychological functioning in children with minimal brain dysfunction receiving d-amphetamine.

Previous Serial Number: Same

Principal Investigator: Paul H. Wender

Other Investigators: Monte Buchsbaum

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.1
Other:	0

Project Description:

Objectives: To compare neurological and psychological functioning of children with minimal brain dysfunction and normal children and to evaluate the changes produced in the former group following treatment with d-amphetamine.

Methods Employed: The study population consists of a group of normal children and a group of children with minimal brain dysfunction who have manifested a clear-cut clinical improvement following treatment with d-amphetamine.

Both groups will be evaluated to assess positions and changes along the following dimensions: (1) average cortical evoked response, and (2) field dependence.

Children with minimal brain dysfunction are characterized by inattentiveness and stimulus boundedness, both of which appear to change following successful treatment with d-amphetamine. These characteristics (and change in them) may be associated with performance on certain tasks; particularly the Rod and Frame test, and the Petrie task, and in average cortical evoked response to sensory stimuli. It is the purpose of this project to determine if correlates exist between the clinical condition, response to treatment, and test performance.

Major Findings: Certain parameters of the evoked response discriminate normal and minimally brain dysfunctioned children with a diagnostic discrimination of approximately 65%. Children who respond favorably to amphetamine treatment differ from non-responders in the direction of their response.

Significance to Bio-medical Research and the Program of the Institute: Minimal brain dysfunction is an extremely common syndrome in the pre-adolescent population, occuring in perhapys 5% of that population. Its neurophysiological basis is not understood and its diagnosis cannot be made with accuracy. This project may contribute to an understanding of its etiology and to more accurate diagnostic technique.

Proposed Course of Project: To date, 25 children with minimal brain dysfunction and 55 controls have been tested. Data analysis has been completed and a summary article will be published this year.

Honors and Awards: None.

Publications: None.

Serial No. M-P-C-(C)-43
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Lithium as a therapeutic agent in hyperkinetic behavior disorders of childhood.

Previous Serial Number: Same

Principal Investigator: Paul H. Wender

Other Investigators: Ronald O. Rieder, James Weiss, Monte Buchsbaum, Theodore Zahn

Cooperating Units: Office of the Chief, Laboratory of Clinical Science

Man Years:

Total:	2.2
Professional:	2.2
Other:	0

Project Description:

Objectives: To compare the physiological, neurological, and behavioral effects of lithium carbonate and dextro-amphetamine sulfate in single blind trials on a hospitalized population of children with the hyperkinetic syndrome who have been unresponsive to amphetamines and phenothiazines.

Methods Employed: Subjects ages 6 to 14 diagnosed as hyperkinetic who have failed to respond to conventional drugs. The subjects are admitted to the NIH. During their first week in the hospital, the children are screened for cardiac and thyroid abnormalities, have routine urinary 17-hydroxy steroid levels taken, and have baseline evoked potentials and autonomic reactivity studies completed. They are placed on a placebo at this time and baseline nurses' behavioral ratings and teachers' ratings at NIH are obtained. During the second week, the children are given dextro-amphetamine sulfate (not to exceed 30 mg. q.o.d., p.o.) and again, 24 hour urines, physiological measures, and teachers' and nurses' behavioral check lists are obtained. During the third and final week, the children are given lithium carbonate (not to exceed 1200 mg. p.o., q.o.d.), daily lithium blood levels are drawn, and the urines are obtained. As the child reaches the therapeutic dose level of between 0.8 mEq. per liter and 1.2 mEq. per liter, he is discharged to home and is followed on a weekly basis in the NIH Outpatient Clinic. Two weeks following the child's first blood level of at least or more than 0.8 mEq. per liter, the third set of physiological studies are done on an outpatient basis.

Because of the favorable effects of the small NIH classroom, frequent one to one nursing, and the strict structured environment of the ward, it is difficult to completely evaluate any possible improvement seen in the hospital. The post-hospitalization period is then used as a true single blind study and behavioral check lists are distributed to the child's teacher and the child's own parents. It is one of the major objectives of this project to establish both the safety and efficacy of lithium carbonate treatment in children outside the hospital.

Major Findings: None. To date ten children have been treated. It appears that these children handle lithium very well and that safe blood levels can be reached and stably maintained without difficulty. Data on the effectiveness of lithium appeared to have no therapeutic effect in the population.

Significance to Bio-medical Research and the Program of the Institute: The hyperkinetic syndrome of childhood is an extremely common syndrome in the preadolescent population, but only one-half to two-thirds of these children respond well to current medication. Recent reports by Annell and Dyson have suggested that lithium carbonate may assist some of these formerly unresponsive children. Both reports tend to stress subgroups of the hyperkinetic population, i.e., Dyson has worked with offspring of manic depressives and Annell's sucess is limited to borderline psychotic adolescents. Furthermore, lithium carbonate treatment may be a less toxic drug than dextro-amphetamine sulfate when given over several years' time.

Proposed Course of Project: Data collection has been completed. Data analysis is completed.

Honors and Awards: None.

Publications: A paper entitled Lithium in the Treatment of Hyperactive Children, is now in press. It is to appear in The Archives of General Psychiatry.

Serial No. M-P-C-(C) - 44

1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Individual Differences in Eye Movement Search Patterns.

Previous Serial Number: Same

Principal Investigator: Monte Buchsbaum

Other Investigators: Richard Coppola, James Bryan, Stephen G. Landau and Lyman C. Wynne.

Cooperating Units: Adult Psychiatry Branch, NIMH
Section on Technical Development, MHIRP

Man Years:

Total:	2.0
Professional:	1.6
Other:	.4

Project Description:

Objectives: Development of a computer controlled, interactive, real time eye movement detector; study of individual differences in eye movement with regard to search patterns, information input and feedback; exploration of eye movement characteristics in normal and various psychopathologic groups.

Methods Employed: The apparatus recently developed by this project utilizes a MacWorth eye movement camera stand and a television camera. The corneal reflection of a visible light source is transmitted through an optical system into a television camera. The cartesian coordinates are determined from the video signal by analog devices, thus yielding the eye's point of regard at any given time. A process control computer (LINC) is used to generate visual displays and record the analog record data. A data editing system allows calibration of the analog inputs and conversion of the data to the screen coordinates. The program then outputs the duration and location of each successive eye fixation.

The resultant system provides for the first time an on-line process control eye movement recorder and display system. Prior to this development individual difference studies have been prohibitively costly in terms of time required for data analysis. For instance, hand analysis of 10 seconds of eye movements recorded on motion picture film would require four hours to prepare before it could be used as computer input. This stage of data analysis is now completely eliminated and the average subject is run on 20 such 10-second trials. The system has been dramatically upgraded this year with the introduction of a silicon matrix vision tube with low noise levels, which permits cleaner records and easier adjustment of subjects. In addition the LINC computer has been interfaced with our medium sized SEL system, allowing rapid statistical analysis of the eye movements. A group of Markov analysis programs was written and utilized in describing the data.

Experimental Methods: Fifteen normal subjects have been tested on two separate occasions. Each subject viewed a computer display of the outline of two squares; one of which was always the same size and the other a variable size from one presentation to the next. He was given the task of ascertaining whether the variable square was larger or smaller than the standard. Sixty twin pairs (Mz and Dz) and 10 schizophrenic subjects have also been tested.

Results: The reliability of individual differences in patterns of eye fixation during a size estimation task were studied in normal adults, each tested twice. The most stable measures not specifically related to the stimulus configuration appeared to be the number of fixations per unit time; measures related to dispersal of looking were unreliable. Data on where the subject looked, the duration of each fixation and the distance between fixation points appeared to follow 1st order Markov processes and the transitional probabilities appeared to be individual characteristics stable over time.

A detailed analysis of the accuracy of the television camera eye movement device has been made. The reproducibility of the XY coordinates is less than 0.5% across a 5 second eye movement data collection run. The absolute accuracy of the XY position determination appeared to be of the same order of accuracy. Distribution of the duration of eye fixations has revealed that eye movements down to about 140 to 160 msec in length do not differ in the character of the starting and stopping of the eye; thus despite some theoretical claims to the contrary, bona fide saccades do appear with durations of fixation under 200 msec. In a free scanning environment a variety of saccadic and smooth pursuit movements appear to be mixed together as the subject solves the perceptual problem presented.

Significance to Biomedical Research and the Program of the Institute: Eye movements are important indicators of information input strategies and may therefore reflect psychophysiological styles of information search. The possibility of using this and other objective, physiological measures to better distinguish psychiatric patients is central to psychophysiological research.

Proposed Course of Project: Characterization of eye movement parameters as 1st and 2nd order Markov chains has suggested a conceptual model of a possible psychophysiological information search strategies--individuals with independent eye movements (0 order Markov process, transitional probabilities independent of current state) lack any overall information gathering strategy or fail to change strategies after inputing relevant information. Further work is planned exploiting the Markov analysis more fully. These data analysis techniques will be applied to the twin and schizophrenic data sample.

Honors and Awards: None.

Publications:

Buchsbaum, M., Pfefferbaum, A., and Stillman, R. Individual differences in eye-movement patterns. Perceptual and Motor Skills, 36: 895-901, 1972.

Bryan, J. S., Pfefferbaum, A., Buchsbaum, M., Carman, G. M., and Stillman, R. C. Data acquisition system for precision eye fixation studies. Behavior Research and Instrumentation, in press.

Serial No. M-P-C-(C)-45
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The offspring of schizophrenics: markers of a schizophrenic disposition.

Previous Serial Number: None

Principal Investigator: Ronald O. Rieder

Other Investigators: David Rosenthal, Paul Wender

Cooperating Units: PHS-NIH-NINDS-PRB

Man Year:

Total:	2.5
Professional:	1.7
Other:	.8

Project Description:

Objectives: To discover differences between the offspring of schizophrenics and controls, at an early stage in life, which may represent a predisposition to develop schizophrenia later in life.

Methods Employed: This study has utilized the data previously collected by the National Institute of Neurological Disease and Stroke, Perinatal Research Branch, in their "Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood." In that project, some 55,000 pregnancies and the offspring were studied, with the 7-year follow-up now being completed. About 900 of the mothers or fathers had had a psychiatric hospitalization, and of this group, we took 220 who were studied in the Boston area to use for our study. We gathered the records of their psychiatric hospitalizations, and over the past year we proceeded to diagnose these subjects. We diagnosed 150 parents, and assigned a diagnosis of schizophrenia to 67 of these. The 150 families produced a total of 210 children who had been part of the NINDS study, and 93 of these were the offspring of schizophrenics. Taking the families containing a schizophrenic parent, we selected a matched control group, and began comparing the findings for our two groups on the investigations which the NINDS-PRB had performed.

Major Findings: There is an increased rate of fetal and neonatal death among the offspring of schizophrenics as compared with controls. Some of these deaths were associated with neurological malformations. Reasons for the deaths were not found at autopsy.

Significance to Bio-medical Research and the Program of the Institute: This work follows the work of this Laboratory on the genetics of schizophrenia. We need to find how the genetic factor underlying schizophrenia clinically manifests itself if we are to effectively intervene or to do biochemical or physiological investigations of the development of schizophrenia.

Proposed Course of the Project: During the next year we will investigate the data which has been collected on these offspring of schizophrenics. This includes serial neurological exams, psychological exams, and language exams.

Honor and Awards: None

Publications: None

Serial No. M-P-C-(C) - #46
1. Laboratory of Psychology
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychophysiological Changes During the Menstrual Cycle.

Previous Serial Number: M-P-C-(C) - #17

Principal Investigator: Theodore P. Zahn, Ph.D.

Other Investigator: Betsy Little (Guest Worker)

Cooperating Units: None

Man Years:
Total: .6
Professional: .1
Other: .5

Project Description:

Objectives: To study autonomic changes through the menstrual cycle. To study the relationships between autonomic changes and changes in time estimation, reaction time, and mood throughout the menstrual cycle. To elucidate the mechanisms responsible for the physiological changes seen in women, progesterone is being administered to men.

Methods: For women, a short experimental procedure is used every day for each subject for a complete cycle. Five autonomic measures are used (finger pulse volume, heart-rate, respiration, skin temperature and skin resistance) in three situations:

(i) giving the subject moderate intensity tones to listen to
(ii) asking the subject to estimate 5-sec. intervals
(iii) giving the subject a simple reaction-time situation where she has to lift a key as quickly as possible on hearing a tone.

For men, the same testing procedure is used as for women. They are tested four days a week for four weeks. During one week (double blind) they receive 50 mgm of progesterone per day. During the remaining three weeks they receive a placebo.

Both subjects and experimenter fill out a mood scale daily.

Major Findings:

(a) With the women: A preliminary examination of the data shows that there are very striking baseline shifts during the cycle. Much higher skin resistance is seen 5-7 days premenstrually, than at other times of the cycle. There is also a premenstrual peak in heart rate which seems to occur just before the flow begins. A considerably longer GSR recovery rate is seen at about the 3rd or 4th day of the menstrual flow with the greatest occurrence of non-specific responses just before ovulation. Positive feelings are strongest just before ovulation, weakest in the premenstrum, while negative feelings flourish at this time. Further analysis of the data will be carried out and the mechanisms responsible for these changes explored.

(b) With progesterone administration to men: Progesterone appears to increase skin resistance in men as it does in women. It is still unclear whether progesterone withdrawal results in heart-rate increase. Psychologically, the men admit to being more tired during the days after progesterone withdrawal.

Significance to Biomedical Research and the Program of the Institute: This study has helped to clarify which of the two hormones, estrogen, or progesterone, is responsible for the negative feelings and physiological changes that occur in the second half of the menstrual cycle.

Proposed Course of Project: Further collection of data on normals and possibly in depressive and schizophrenic patients as well.

Honors and Awards: None.

Publications: None.

Serial No. M-P-C(C) - #47
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychophysiological Concomitants of Minimal Brain Dysfunction in Children.

Previous Serial Number: M-P-(C) - #17

Principal Investigator: Theodore P. Zahn

Other Investigators: Paul H. Wender, Betsy Little (Guest Worker)

Cooperating Units: None

Man Years:

Total:	1.9
Professional:	.8
Other:	1.1

Project Description:

Objectives: To compare MBD children with normal children in autonomic functioning. To assess the effects of drug therapy. To predict MBD children's response to drug therapy.

Methods Employed: Peripheral measures of autonomic functioning, namely skin resistance, heart rate, respiration, skin temperature, and pupil size are recorded during two sessions in which children are exposed to mild auditory and visual stimuli, perform reaction time tests and perform a cold pressor procedure. Parents are given questionnaires relating to child rearing attitudes and practices, and their child's behavior.

Subjects are children from the Washington area with a diagnosis of MBD under treatment by a psychiatrist. Controls are from a nearby elementary school and have been chosen by their teachers as not having significant behavior problems. The patients are tested both on and off amphetamine therapy.

Comparison of autonomic base levels, responsivity to meaningless and meaningful stimuli and variability will be made for normal vs. MBD children, for MBD children on and off amphetamines, and between MBD children who respond successfully to the drugs (as judged by their psychiatrist) and those who do not.

A small number of MBD patients who did not respond to amphetamines were given a clinical trial of lithium. Those subjects have been tested both on drugs and off drugs.

Major Findings: Lithium produced an increase in heart rate and respiration rate in most subjects. However, the only two patients to show a clinical improvement on lithium were also the only two to show no heart rate increase. Further results with the larger group await completion of testing and data analysis.

Significance to Biomedical Research and the Program of the Institute: The reports in the literature suggest that MBD children may be hypoaroused. It is of great importance to test this, since, if true, it would help to explain the "paradoxical" effect of amphetamines. This study should suggest the extent of autonomic involvement in MBD, and hopefully will have some predictive value in determining which children will respond successfully to amphetamines.

Proposed Course of Project: Continued data analysis.

Honors and Awards: None.

Publications: Greenhill, L., Rieder, R. O., Wender, P. H., Buchsbaum, M., and Zahn, T.P. Lithium in the treatment of hyperactive children. *Archives Gen. Psychiat.* In press.

Serial No. M-P-C-(C) - #48
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS-HSMHA - NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Autonomic Functioning in MZ and DZ Twins.

Previous Serial Number: M-P-C-(C) - #17

Principal Investigator: Theodore P. Zahn

Other Investigators: Monte Buchsbaum

Cooperating Units: None.

Man Years:

Total:	.3
Professional:	.1
Other:	.2

Project Description:

Objectives: To determine the relative importance of genetic vs. environmental factors in various aspects of autonomic functioning. To determine the relationships of autonomic nervous system activity to personality, task performance and electrocortical functioning.

Methods Employed: Peripheral measures of autonomic functioning, namely skin resistance, heart rate, finger pulse volume, respiration, and skin temperature are recorded during two sessions which include rest periods, a series of mild tones, a reaction time task and mental arithmetic. The subjects are 30 MZ and 30 DZ twin pairs equally male and female. Some opposite sex DZ pairs have also been tested.

Intraclass correlation will be the primary method of analysis. It will be based on base levels, responsivity to non-demanding and demanding stimuli, variability and response specificity.

Major Findings: None, as yet.

Significance to Biomedical Research and the Program of the Institute: Aside from the inherent interest in the basic question of the heritability of autonomic indices and patterns, the data from this project will be used to help interpret the earlier findings from a similar study on schizophrenic MZ twins (M-P-C-(C)-#17).

Further Course of Project: Continued data analysis. Correlation of autonomic data with personality, behavioral and electrocortical data on the same subjects.

Honors and Awards: None.

Publications: None.

Serial No. M-P-D-(C)-30
1. Laboratory of Psychology
2. Section on Early Learning and Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Stimulus Conditions, Infant Behaviors, Caretaker-Child Interaction, and Social Learning in Diverse Child-Rearing Environments

Previous Serial Number: Same

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Cooperating Units: Department of Psychology, Hebrew University of Jerusalem; Social Science Statistical Center, University of Maryland Baltimore County

Man Years:

Total:	.40
Professional:	.20
Other:	.20

Project Description:

Objectives: In this continuing project, diverse behaviors which connote the quality of adaptation and social responsiveness of infants to their environments are being related to stimulus conditions which represent opportunities for adaptive and social learning in early life. Within each of four child-rearing environments, our aims have been: (a) to describe the typical day for the infant, including a catalogue of caretaking schedules, the types of stimuli provided (primarily those provided through interaction with caretakers), and the types and frequencies of behaviors of the infant; (b) to determine the sequential contingencies between environmental stimuli and infant responses insofar as they represent opportunities for learning; and (c) to consider specific performance issues, such as the infant's response to short-term separation from a caretaker to whom he has become "attached" (e.g., his mother), and her responses to his subsequent initiations. It is thought such data can provide a fruitful basis for examining the utility of the concepts available for conceptualizing the child's early experience with his environment.

Methods Employed: Subjects (Ss): Samples of Ss were drawn from the infant population of Israel. The sample currently emphasized consists of 108 Ss in four age groups (8, 16, 24, and 32 weeks) from four child-rearing environments [the Residential Institution, the Kibbutz, the Only-child and the (Youngest-child of) Multiple-child Middle-class Urban family]. Each S was observed in his natural setting by a trained woman observer for two half-days, providing data to represent one complete day of his life.

Methods Employed (Continued):

A codified observation procedure was used. It involved reliable coded observation categories for: (a) background events (e.g., mother enters, caretaker talks to visitor, caretaker attends to neighboring child); (b) setting (e.g., feeding, sleeping, bathing); (c) S's behaviors (e.g., smiles, vocalizes, follows, manipulates); (d) the various stimuli provided by (behavior of) the environment, particularly those of caretakers toward S (e.g., mother approaches, caretaker picks up S, father tickles S, caretaker smiles); (e) the sequential contingencies comprising interactions between the infant and his environment; (f) S's gross activity level (e.g., changes position off and on, mouths). Behaviors (but not their durations) were scored as they occurred during the observation period.

Major Findings: During the past year, we have continued our concentration on caretaker-infant interchange, in particular comparisons, by age or environmental group, of the correlation coefficients between three infant and three mother (or caregiver) behavior classes during the observation day. Specifically, correlations were examined between the Child's visual fixation, smiling, and vocal behaviors, and the Mother's (or Caregiver's) fine contact, smiling, and talking behaviors. These correlations were grouped within caregiving settings, within purely-social settings, and between caregiving and purely-social settings. This correlational analysis that provided the dependent variables of this study was performed upon the gross features of the dyadic interaction chains comprised of the responses of mother (or caregiver) and her infant, ignoring the concurrent and sequential features of those interchanges. It is therefore conceived to be a pre-sequential *in vitro* analysis that can identify some interesting child-rearing group and age group differences in the gross features of interaction patterns. The magnitudes of each mother- (or caregiver-) infant behavior correlation coefficient was compared for the environmental groups or, independently, for the age groups. The statistically-reliable findings follow:

1. Correlation coefficients between mother and infant interaction behaviors were more often higher in kibbutzim than in youngest-child or only-child urban middle-class families.

2. In kibbutzim, the correlations between interaction behaviors of mothers and their infants were more often higher than were those between interaction behaviors of professional caregivers (metaplot) and the same infants.

3. The correlations between interaction behaviors of kibbutz caregivers and their infants were more often higher than were those between residential institution caregivers and their infants.

4. The correlations between interaction behaviors of mothers and their infants, and of caregivers and theirs, were more often higher when the

Major Findings (Continued): infants were 8 months old than when the infants were 2 months old.

In an attempt to gain an understanding of some possible bases of these results, the intercorrelation-coefficient magnitude patterns were examined within-infant behaviors and within-mother or -caregiver behaviors by environmental groups and by age groups. It was found that these patterns paralleled fairly closely the correlation coefficient patterns found between mother or caregiver and infant behaviors. Hence, it is prudent to emphasize the homogeneity of all magnitude patterns characterizing the *in vitro* correlation coefficients derived from mother/caregiver-infant interaction, whether adult-infant, infant-infant, or adult-adult. This homogeneity may simply result from the fact that all the coefficients are derived from the very same behavior-data base. In this context, it could be concluded from the product-moment correlation analysis that the interaction of kibbutz boys, and of 8-month infant boys, with their mothers or caregivers is more homogeneous than is the interaction of mother- or caregiver-infant dyads within the urban middle-class youngest child or only child family groups or within the 2-month-old group.

This higher correlation pattern characterizing interaction in the kibbutz environment, compared to those of youngest children and only children, may be due simply to the possibility that there is less of a distinction in kibbutzim between caregiving and purely-social settings. The greater number of high between-setting kibbutz correlations found would support this possibility, but the higher coefficient magnitude patterns found for kibbutz mothers than for kibbutz caregivers would not.

While considerable empirical leverage has been attained on the difference in role between mother and caretaker in the kibbutz environment, between caretakers in the kibbutz and institution, as well as between kibbutz and urban family mothers, the next step is to apply to the data techniques that better summarize the concurrent and sequential facets of mother-infant interchange *in vivo*; techniques that do not give up as much of the structural detail as was discarded in the analyses reported here.

Scientific Significance to the Program of Mental Health Research: Watching-scanning, smiling, vocal, and similar adaptive behaviors constitute key human response systems, for they mediate (and hence can also index) much of initial adaptive and social learning and development of the infant as well as his subsequent patterns of social-interaction. A developmental picture of the early course of these behavior systems can be of considerable use in itself, but can also provide a context for understanding the nature of the outcomes of different conditions of child-rearing. These conditions can be assumed to provide differential varieties and amounts of stimulation, and hence varying opportunities for social learning. At the same time, this investigation can provide data on an issue that has become increasingly salient in parent-child relations: how the child's behaviors can affect the parent's or caretaker's behaviors in relation to him.

Proposed Course of the Project: Reports from this project have been delayed by our move to new quarters and our need to tool up to begin a new research program there. Our plan is regularly to issue reports from this project for publication. It is anticipated that several such reports will be completed during the coming year.

Honors and Awards: None

Publications: None

Serial No. M-P-D-(C)-34
1. Laboratory of Psychology
2. Section on Early Learning and Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Contextual Determinants of Stimulus Power

Previous Serial Number: Same

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Other Investigators: None

Cooperating Units: University of Maryland Baltimore County

Man Years:

Total:	.30
Professional:	.15
Other:	.15

Project Description:

Objectives: A great variety of preceding or concurrent contextual conditions potentially exist which could determine the power of a focal stimulus in evoking, cueing, or reinforcing behavior. Their effects often cumulate. Our aim has been to survey the range of contextual factors that can qualify stimulus efficacy, to make a preliminary categorization of them, and to consider some underlying issues in their operation.

Methods Employed: In the empirical portion of this program, some key contextual factors have been manipulated. For instance, deprivation and satiation conditions for a social stimulus class were effected by varying the availability of a social stimulus to subjects in a period immediately preceding a test for the effectiveness of that stimulus. A further aim has been to implement different maintenance patterns of prior stimulus availability.

Major Findings: Data from diverse sources have been used to identify classes of contextual factors. In this frame and on the basis of our earlier empirical studies, it was concluded that the function of a stimulus on a particular occasion, in evoking, cueing, or reinforcing behavior, may be enhanced or decreased by the manipulation of the context of stimulus provision. Moreover, even the direction of the effects of a stimulus on behavior may be determined by the stimulus context. That is, whether a given stimulus evokes an approach or an avoidance response will often depend entirely on the context of its provision.

Major Findings (Continued):

Our earlier work with children has shown that the power of a reinforcing stimulus in learning is an inverse function of their prior satiation for it, specifically of the number of times the children have been exposed to the same stimulus in a setting immediately prior to the learning task. To determine if this function would hold also for stimuli of high salience provided prior to the learning task, as it does for stimuli of low salience, an experiment was carried out with 50 grade-school boys. High saliency was implemented during the treatment condition by providing the stimulus word good clearly contingent on a particular response. The results indicated that a satiation function applies as well to stimuli of high saliency, permitting a more general conclusion about reinforcing-stimulus satiation.

Scientific Significance to the Program of Mental Health Research: The efficiency of human learning and performance depends on the effectiveness of the available stimuli in each of their roles. Hence, the identities of contextual setting conditions can provide an important key for understanding the acquisition and performance of child behavior systems, particularly in natural settings. The principles identified should also bear on such conceptions as childhood "privation" as they suggest that effective stimulation depends not only on the type and number of stimuli available but also on the contexts of their provision as well.

Proposed Course of the Project: Our plan is to continue experiments in this problem area.

Honors and Awards: None

Publications: None

Serial No. M-P-D-(C)-42
1. Laboratory of Psychology
2. Section on Early Learning and Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Evaluation of Concepts Employed for Early Learning and Development

Previous Serial Number: None

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.30
Professional:	.15
Other:	.15

Project Description:

Objectives: To analyze the roles in theory and research of focal concepts employed in the area of early adaptive and social learning and behavioral development, and thereby to provide a basis for facilitating and evaluating researches in those sectors. Included are the concepts of development, critical period, environmental stimulation, drives and motivation, stimulus privation, deprivation, and separation, attachment and dependence, imitation and identification, and vicarious-reinforcement and observational learning.

Methods Employed: The moment-to-moment details of the interchange between stimuli and responses are relevant to many process theories of learning and development. However, researches done under the aegis of such theories have almost routinely employed global concepts for environment and behavior (e.g., traits) that only summarize through lengthy time spans the occurrence of either stimuli or responses but not both facets of the (S-R) interchange. The research variables based on these global concepts are therefore often remote from the level of analysis required by the process theories that have spawned them. Our approach to human social learning has proceeded as a detailed functional analysis of stimuli and responses, their interchange at a particular moment, and the sequences of interaction across successive moments (i.e., the stimulus-response chain). Thus, the conditioning concepts we have employed order environmental operations that effect systematic and (usually) reversible changes

Methods Employed (Continued): in observable behaviors. The approach employed is open to the addition of new concepts as required, and to the differentiation and refinement of the concepts in current use.

Major Findings: Progress was made during the year in evaluating the efficacy of the key attachment and dependence concepts in ordering the behavioral phenomena for which they were devised, and in relating them to our observations in child-rearing settings. The conception of these terms was further developed as abstractions for classes of functional relationships involving the positive stimulus control over a wide variety of an individual's responses by stimuli provided either by a class of persons (dependence) or by a particular person (attachment). Potential indicators for those concepts have been surveyed under this conception for their acquisition and maintenance. Indices were surveyed for other cases as well, including those involving a) different approaches to the origins of attachment and dependence, b) different bases of distinctions between the concepts, and c) diverse assumptions underlying cognitive-ethological and learning approaches to human attachment and the mother-infant relationship. As dependence and attachment are conceived as gross abstractions, many types of behaviors (e.g., approach, preference, approach in the context of avoidance, disorganization behaviors) could be used to index each concept in different contexts. Bases for index selection under different research strategies and tactics have been considered.

Scientific Significance to the Program of Mental Health Research: Clarification of the purpose and utility of concepts such as those examined should lead to more efficient mental health research and application. The role of response acquisition in various complex personality processes that underlie such concepts, and distinctions between motivation and learning concepts, can be delineated more explicitly and parsimoniously. On this basis, research and its applications in the area of the psychological development of the normal child should be facilitated.

Proposed Course of the Project: The need for this project is a continuing one, as concepts like the ones being analyzed are in common use in the field at large and serve important functions in our research program.

Honors and Awards: None

Publications:

Gewirtz, J. L. Attachment and dependence: Some strategies and tactics in the selection and use of indices for those concepts. In T. M. Alloway, L. Krames, and P. Pliner (Eds.), *Communication and Affect*. New York: Academic Press, 1972. Pp. 19-49.

Gewirtz, J. L. Attachment, dependence, and a distinction in terms of stimulus control. In J. L. Gewirtz (Ed.), *Attachment and Dependency*. Washington, D.C.: Winston, 1972. Pp. 139-177.

Publications (Continued):

Gewirtz, J. L. On the selection and use of attachment and dependence indices. In J. L. Gewirtz (Ed.), Attachment and Dependency. Washington, D.C.: Winston, 1972. Pp. 179-215.

Serial No. M-P-D-(C)-43
1. Laboratory of Psychology
2. Section on Early Learning and Development
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mutual Learning Implications of Mother-Infant Interaction

Previous Serial Number: NONE

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Other Investigators: Elizabeth Boyd, Ph.D.

Man Years:

Total:	2.00
Professional:	1.50
Others:	.50

Project Description:

Objectives: Theoretical and empirical approaches to child behavioral development have been carried out for the most part under the ubiquitous, but one-sided, assumption that parent behaviors are the main influencers of child behaviors, and that the influence the child's behaviors may have on his parent's behaviors plays a relatively minor role in the child's socialization. In this frame, learning approaches have been concerned with the conditionability of selected behaviors of the infant by actual or simulated aspects of the social environment. During the past decade, however, the issue of the direction of socialization effects has become increasingly focal for diverse theories of early child development and approaches to mother-infant interaction. Beginning in the late 1950s, our approach to this matter has been conceived in a learning framework, the central substantive assumption being that at the very same time as the mother may be conditioning (reinforcing) her infant's behavior(s), her infant may be conditioning her behavior(s). This conception provides a basis for beginning a systematic, experimental analysis of the sequential features of parent-infant interchanges.

Methods Employed: In this conceptual context, three designs have been formulated to provide bases for the studies planned as first steps to answer two concurrent questions: 1) Can infant behaviors (actual or simulated) function to modify (reinforce) aspects of mother behaviors, and which infant behaviors so function? 2) What is the effectiveness of selected mother behaviors in modifying features of particular behaviors of her infant? The plan is to carry out these studies mainly under laboratory conditions, with occasional probes of such mother-infant influence patterns

Serial No. M-P-P-(C)-6
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Investigation of Some Formal Characteristics of Speech

Previous Serial Number: Same

Principal Investigator: Donald S. Boomer, Ph.D.

Other Investigators: Allen T. Dittmann, Ph.D.

Cooperating Units: None

Work on this project is temporarily suspended and is being held in abeyance.

Serial No. M-P-P-(C)-22
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of Potentially Creative Scientists: Personality Characteristics Associated with Creative Performances.

Previous Serial Number: Same

Principal Investigator: Kenneth Burgdorf, Ph.D.

Other Investigator: None

Cooperating Units: None

This project has been discontinued.

Serial No. M-P-P-(C)-41
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Survey of Literature on Emotional Communication.

Previous Serial Number: Same

Principal Investigator: Allen T. Dittmann, Ph.D.

Other Investigators: None

Cooperating Units: None

This project has been completed.

Publication:

Dittmann, A. T.: Interpersonal Messages of Emotion. New York, Springer Publishing Co, 1972, 232 pp.

Serial No. M-P-P-(C)-42
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Precocious Science Students in Psychiatric Treatment: A Longitudinal Study

Previous Serial Number: Same

Principal Investigator: Kenneth Burgdorf, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.00
Professional:	.60
Other:	.40

Project Description:

Objectives:

1. To describe the experiences of a large group of high aptitude science students who received psychiatric treatment during college.

2. To examine the nature of these students' difficulties and the manner in which they developed.

3. To assess the impact of current modes of psychiatric treatment on the functioning of these students.

Methods Employed:

1. A wide variety of measures - including indices of personality traits, self-perceptions, parent-child relationships, interests, activities, achievements, etc. - were collected on a nationwide sample of 756 precocious science students during their senior year of high school.

2. Similar data were collected at two subsequent points in time, after the freshman year of college and after the first year of graduate school (i.e., 5 years after high school graduation). A total of over 150 colleges and universities were represented in this sample.

3. In the post-college investigation, subjects were asked whether or not they had received professional assistance for personal or emotional problems

while at college and, if so, to describe the form and duration of the assistance which they received. Analyses focused on the group of 95 students who reported that they had received 5 or more sessions of psychotherapy.

Major Findings:

1. The proportion of students who expressed concern about emotional well-being at college was unusually high. Nearly half of the sample reported that they had seriously considered entering treatment, and 23% of the sample reported having received some form of psychiatric treatment.

2. Specific problems of adaptation to the college environment appeared to be the primary source of motivation for help-seeking. Loneliness, dissatisfaction with grades, and a general feeling of being "out of place" at their colleges were prominent among the patients' complaints.

3. Of the students who received psychiatric treatment at college, approximately 2/3 did not show signs of significant emotional difficulties or problems in adjustment prior to college.

4. Two patterns of student-college interaction were observed: (a) Among the students who showed signs of emotional impairment prior to college, those who entered highly selective colleges (Harvard, MIT, etc.) were very likely to experience adjustment problems and to seek assistance. Those who entered less selective colleges tended not to experience problems in adjustment, not to enter treatment, and to improve spontaneously over time. (b) Most unimpaired students entered colleges with highly competitive, highly structured academic programs, and majored in science. Those who evidenced a complex of personality traits which has been found associated with high-level creative performance in adult samples were much more likely than those who did not evidence such traits to adapt poorly to their college environments and to seek assistance.

5. Approximately 85% of the treatment group received some form of psychotherapy. No evidence was found that this form of intervention produced an alleviation of problems or a reintegration of functioning. On the contrary, the functioning of students who received psychotherapy tended to decline over time. This decline was not confined to any one aspect of functioning but was evident on measures of change in personality, self-perception, social and family relationships, physical health, academic performance, and career development. Approximately 70% of the patients in this group evidenced such changes.

6. Approximately 5% of the students who sought professional assistance received educational-vocational counseling. This was the only treatment subgroup to produce evidence of beneficial treatment impact. All of the students in this group showed signs of improvement subsequent to treatment.

Scientific Significance to Mental Health Research Programs:

Historically, the emphasis in college psychiatry has been on the development and staffing of programs designed to make psychotherapy available to large numbers of students. It appears that this goal has been achieved at many colleges, and perhaps overachieved. At the present time, it may be that too many rather than too few college students are given psychotherapy. Consideration of the predispositions, problems, and educational situations of the students represented in this study suggests that educational-vocational counseling was probably the treatment of choice in many cases. A priority need at this time is for the development of procedures to discriminate effectively between those malfunctioning college students who are likely to benefit from psychotherapy and those who are more likely to respond favorably to alternative methods of therapeutic intervention. To meet this need, further research detailing the responses of different kinds of students to different modes of intervention is required.

Proposed Course of the Project:

A monograph describing the findings of this study has been prepared for publication.

Honors and Awards: None

Publications: None

Serial No. M-P-P-(C)-43
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Test and Performance Measures of Creativity in Science

Previous Serial Number: Same

Principal Investigator: Kenneth Burgdorf, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.08
Professional:	.04
Other:	.04

Project Description:

Objectives:

1. To assess the construct and predictive validities of six well-known tests which are alleged to measure "creative ability" in science and which have frequently been used as operational definitions of creativity in substantive research.

2. To assess the construct and predictive validities of a simple test devised by the senior investigator to illustrate a personality-oriented approach to the measurement of creativity.

3. To develop an empirically-keyed creativity measure which would be suitable for use in substantive research.

Methods Employed:

1. Subjects were 131 male high school seniors who: (a) had indicated an interest in becoming professional scientists, (b) had demonstrated high levels of knowledge about basic scientific facts, principles, and methods, and (c) had produced at least one major research project in a field of science. The rationale was that, unless the sample were restricted to subjects who possessed high levels of research-relevant knowledge, opportunity, and motivation, individual differences in research creativity could not be interpreted as indicative of individual differences in creative ability and strong associations between valid creative ability tests and criterion

measures could not be anticipated.

2. Two criterion measures were formed. One involved assessments by prominent scientists of the creativity of subjects' research projects. Reasoning that scientific research is an intrinsically creative activity and that any indication of research quality might be a useful index of manifest creativity in this sample, a second criterion was formed by assessing each subject's level of scientific achievement (i.e., national vs. less than national). It was predicted that each test would be found positively associated with at least one criterion.

3. A set of reference variables was obtained. These included measures of achievement and activity in science and in other creative fields, creativity self-ratings, indices of academic achievement, family background, etc. Predictions were advanced regarding the pattern of correlates which the tests and criteria would produce with these reference variables if they are valid.

Major Findings:

1. The criteria were found to possess satisfactory levels of construct validity. They were found associated with one another and with reference variables generally as expected.

2. The creative ability tests were found to possess very little predictive or construct validity. They were found not to be related as predicted to one another, to the criteria, or to the reference variables. The personality test, on the other hand, was found to possess surprisingly high levels of predictive and construct validities.

3. The findings were interpreted as raising questions both about the validity of the ability tests studied and about the utility of the theories which generated them. The personality test findings were interpreted as indicating that a personality-oriented approach to the conceptualization and measurement of creativity is a potentially useful one. It was emphasized, however, that the personality test was designed for illustrative purposes only and was not regarded as a definitive measure of the creative personality.

4. On the basis of these findings, an empirical keying procedure was utilized to develop a creativity measure which would be suitable for use in substantive research. This scale is composed of 29 items from the item pool of the California Psychological Inventory, a general purpose personality inventory. Each of these items was found to be statistically associated with the measure of research creativity which served as a criterion in the earlier study.

5. This creativity scale was shown to possess a high degree of construct validity. It was found strongly associated: (a) with both criteria from the earlier study, (b) with measures of involvement and achievement in science, music, art, and other creative fields, (c) with self-ratings of creativity in these fields, and (d) with measures of personality traits theoretically associated with the concept of creativity - independence of judgment, ego strength, flexibility, cognitive complexity, etc.

Scientific Significance to Mental Health Research Programs:

Creativity has become the subject of much speculation in recent years, much of which involves mental health matters. It has been claimed that some forms of psychotherapy enhance creativity, that creativity is incompatible with mental illness, that certain child-rearing practices and certain kinds of educational environments impede the development of creativity, etc. Before such speculations can be verified or disproven, valid measures of creativity must be found. This project is felt to contribute to this end by demonstrating that several frequently used creativity tests are not valid and by demonstrating the fruitfulness of an alternative approach to the conceptualization and measurement of crearivity.

Proposed Course of Project:

A report of the findings of the first study has been submitted for publication. A report describing the properties of the empirically-keyed creativity scale is in preparation.

Honors and Awards: None

Publications: None

Serial No. M-P-P-(C)-44
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Psychodynamic and Instrumental Learning Models: Implications for Personality Theory and Psychotherapy.

Previous Serial Number: Same

Principal Investigator: Stanley I. Greenspan, M.D.

Other Investigator: None

Cooperating Units: None

This project has been discontinued.

Publication:

Greenspan, S. I.: Joining aspects of psychodynamic and operant learning theories. Int. J. Psychoanal. Psychother. 1: 26-49, 1972.

Serial No. M-P-P-(C)-45
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Developmental Factors in Conversational Behavior

Previous Serial Number: Same

Principal Investigator: Allen T. Dittmann, Ph.D.

Other Investigator: Donald S. Boomer, Ph.D.

Cooperating Units: Green Acres School, Bethesda, Maryland

Man Years:

Total:	1.25
Professional:	1.00
Other:	.25

Project Description:

Objectives:

General: To study the development of adult-type interaction patterns of conversation from the dual standpoints of language comprehension and social feedback.

Specific: To define conversation as a rule-governed social system, and compare children's participation in that system with that of adults.

Methods Employed: Systematic observations of children in natural and laboratory settings, and comparison with norms from adults. The initial observations this year were made at a summer day camp, including a wide range of ages from nursery school to early adolescence. Younger children's talk was seen to be limited to comments on the activities they were immediately engaged in, and to take the form of short bursts of speech. These two features contrast strongly with adult patterns, most of whose conversation is more abstract, that is, concerns past experiences and future plans, and includes longer utterances. The listener in conversation thus serves different functions in the two contrasting age groups. For children the listener is a stimulus for talk in that children talk more with than without an audience, but the listener does not necessarily participate in what the speaker is saying. For adults a listener is necessary for talking to take place (the occasional adult who "talks to himself" is viewed as eccentric at the very least), and the listener must contribute by letting the speaker know how he (the speaker) is doing, that is, how well he is being understood. Thus a working definition of adult-type conversation includes two features: 1) the topic must be removed from the immediate activity to some degree, or be abstract, and 2) the utterances must be of some minimum length, at least two Final Juncture Units (FJUs) in the current definition. The FJU is a rhythmical unit of speech based on the phonemic clause (PC) as it has been studied in projects in this Section, such as the current M-P-P-(C)-6, over the years; an FJU consists of the PC or group of PCs terminated by either rising or falling junctures.

This year a systematic observational method was developed for naturalistic settings and tested in a private school. The data recorded are 1) the number of speaking turns during a 5-minute period; 2) the length of talk per turn in FJUs; 3) the number of listener responses (LRs-like head nods and "M-hm"); 4) the topic, dichotomized as immediate-remote (or concrete-abstract); 5) the participants in the conversation: their number, age, and sex. The data obtained on the first three factors listed are numbers that above lend themselves to recording on a special small hand counter, one of which has been constructed for this purpose by the Section on Technical Development.

Additional data in the laboratory setting were collected using videotape to make records, which are analyzed in the same terms as those noted above, and with control on additional variables.

Major Findings: The observational protocol yields quantitative data directly comparable with those derived from analysis of video taped records in the laboratory, though limited, of course, to the factors that can be included in the naturalistic observations. The method was pretested on 8th grade children in one school setting, and showed that they do occasionally carry on adult-type conversational interaction (the frequency of their conversations can only be evaluated when data are collected from younger children and from adolescents and adults in comparable settings), and that their rate of listener responses lies between that of younger children and adults in the laboratory setting. The rate in a group of children from 6 to 12 years of age studied in the laboratory was .13 LRs per FJU (under .09 for most 6 to 8-year-olds), and .36 for adolescents and adults. The 8th graders observed in this pre-test gave LRs at the rate of .25 per FJU. The total frequency of their conversations per unit of time appeared to be lower than that of adults -- though it must be emphasized that comparison data in a similar setting are not yet vailable.

Data from the laboratory have confirmed the findings of last year on the difference in rates of listener response between children and adults, the contrast becoming sharper with additional data. Refinements in instructions under different experimental conditions have been introduced to begin to explore a number of the parameters of the situation, but no results can yet be reported.

Scientific Significance to the Mental Health Research Program: Conversational behaviors lie at the interface between language and social responsiveness, and open a number of research and practical possibilities ranging from basic research about language learning to educational and diagnostic applications. From the standpoint of language learning, behavior of both speakers and listeners in conversations can tell us more about the development of mastery of both the encoding and decoding processes, as have been studied among adults in this Section for many years. It appears from early results that the acquisiton and reliable use of language arrives at a stage of life far later than much of the past research in language learning has concentrated on. By now there are a number of investigators working on the development of language after age 5 -- and the first review of this literature has just appeared. The traditional tie spoken of by educators between speaking, listening, and reading has had little substance to draw upon in the realm of listening, and so this research has obvious educational possibilities for application.

The development of the social aspect of conversation, that of exchange between two (or more) people, all of whom have specific functions in helping each other keep the process going, also has clear applicability. In developmental diagnosis there are few specific behaviors we are able to point to that might mark a child's progress toward maturity. The extent to which he participates in the rules of conversation can give us one set of specific behaviors to look at -- provided we can obtain age norms for comparison.

Proposed Course of Project: Partly to develop such norms for specified settings, and to explore the parameters of the findings already obtained.

Honors and Awards: None

Publications:

Dittmann, A. T.: Development factors in conversational behavior. J. of Commun. 22: 404-423, 1972.

Dittmann, A. T.: Style in conversation. Semiotica. In press.

Sachs, B. B., Dittmann, A. T., and Sussman, A. E.: Psychological interviewing with deaf persons. J. Rehabil. of Deaf. 6: 140-155, 1972.

Serial No. M-P-P-(C)-46
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Stimulus Intensity Modification: Neurophysiologic and Psychoanalytic Relationships.

Previous Serial Number: Same

Principal Investigator: Stanley I. Greenspan, M.D.

Other Investigators: Monte Buchsbaum, M.D.

Cooperating Units: None

This project has been discontinued.

Serial No. M-P-A-16
1. Laboratory of Psychology
2. Section on Higher Thought Processes
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Study of the Means-end Thought Processes in Human Subjects.

Previous Serial Number: Same

Principal Investigator: Edward A. Jerome

Other Investigators: None

Cooperating Units: Technical Development Section, NIMH, Computer Systems Laboratory, DCRT

Man Years:
Total: 4.00
Professional: 1.00
Other: 3.00

Project Description:

Objectives: It is the purpose of this project to investigate the mental activities that are responsible for resolving problematic situations that occur in the course of the individual's interaction with his environment. Problems often result from imperfect knowledge of the operational regularities of the external systems, and resolution of the difficulty is achieved by competent inquiry into these regularities. In order to be effective, the inquiries must be guided by a set of cognitive principles collectively called heuristic processes. This project has the following objectives: to describe the distribution of heuristic capabilities in various cross-sections of the population, to discover means of facilitating the acquisition of heuristic principles, to find ways of improving the ability to apply such principles, and to participate in the development of a normative theory of effective heuristic structures.

Methods Employed: Standard tests of mental ability are administered for comparison with performances on a variety of computer-controlled system analysis problems developed for this project. A set of 14 related classes of problems called HEPP has been described in previous reports. Two sets of new problems were developed last year for the new SEL 810B real-time computer configuration. The new problems, called ATSEL and ATREC, monitor improved procedures for the selection and reception paradigms of experiments on concept formation. All of these programs implement special controls that were impossible (prohibitively expensive and/or cumbersome) without the support of a high speed process-control computer.

The programs that controlled the procedures referred to below are being organized into a system capable of directing four stages in the execution of psychological experiments: (1) the application of the general design, e.g., randomly or systematically assigning subjects and conditions; (2) giving orientation instructions and testing for comprehension; (3) executing the procedures assigned in (1) and recording the run-time results; (4) collating the data from the assigned sessions and formatting it for input into NIH-DCRT statistical programs. If stage (4) can be interfaced with the central computer, this system could free the investigator to devote his time to planning and interpreting experiments providing he could secure programming support. This work has, however, been seriously impeded by reductions in personnel.

In general, the method used in these studies consists in analyzing responses obtained from patients as they solve a variety of problems that require them to resolve situations in which uncertainty results from not knowing which subset of a specified set of factors is relevant to a desired outcome. When the given set is known to include all possibly relevant factors, two kinds of information about any particular factor is possible: one implies that the factor is relevant, the other implies that it is irrelevant. Sufficient information to control outcomes may be a mixture, in various proportions, of these two types of implication, i.e., the set of factors implied to be relevant may not, in itself, be sufficient, and the set of factors implied to be irrelevant may not, in itself, be sufficient, but the sum of these two sets of implications may suffice to yield the needed information. The difficulty of these tasks is believed to depend partly on the size of the possibility sets and the relations that may be required to exist between relevant factors and partly upon the patient's understanding of these possible relations. The object of research is to discover how cognitive processes are related to various objective properties of enigmas and how these relations can be modified to the advantage of the patient.

Major Findings: An intuitive analysis of the patient's task suggests that it comprises two conceptually isolable components: (1) accessing data that are needed to justify a conclusion and (2) processing or interpreting these data to produce a solution. These processes may occur sequentially or alternately; i.e., data may be accessed with no effort to interpret it until some amount judged appropriate has been stored, or each item of information may be interpreted as it is accessed until a conclusion evolves--of course, various compromises between these extremes may be adopted.

The Section is in process of reporting a study in which college students, working on only moderately complex problems produced disturbingly unsagacious inquiries--they accessed information inefficiently and numerous redundancies impugned their ability to interpret the information they had elicited. However, performance improved with experience; first, the ability to select pertinent inquiries increased, then, interpretation improved. Initially few students integrated the process of interpretation with that of acquisition, but, after solving about 10 problems, more than half of them showed evidence of acquiring the ability to process the data as it was accessed.

Problems governed by the various logical operators traditionally used in this kind of experiment usually exhibit differences in apparent difficulty that are often attributed to unique effects of the logical operators. The results of the present studies have provided some interesting perspectives on this kind of analysis. Adjustment in terms of information-theoretic factors on the pre-and post-objective-solution trials separately were found to account for most of the observed differences in apparent difficulty. This, of course, renders the results consistent with a priori expectations based on objective aspects of the problem spaces involved.

Another experiment, using an alternative method, the reception paradigm, was completed in the present reporting period. It was concerned with the familiar heuristic that recommends that in searching for the variables that are relevant to a given event one should "change only one variable at a time" (conservative focus strategy). It is not generally recognized that this search technique generates several kinds of data--e.g., information about relevance, about irrelevance, or about various mixtures thereof--which, though they may be equally informative objectively, may not be equally useable by a human problem solver. The results of this study, though not completely analyzed, indicate that people use information about irrelevance very inefficiently, the degree of inefficiency being proportional to the complexity of the problem. However, the ability to use information about irrelevance appears to improve with experience.

Scientific Significance to the Program of Mental Health Research: The ability to cope successfully with the unexpected, unfamiliar, and enigmatic situations that repeatedly challenge the individual in the course of his daily life is probably the most important determiner of his mental health and well-being. The efforts of the Section are oriented directly toward improving these particular capabilities; to this end it constructs, organizes, and tests theories of means-end thinking, and develops methods of detecting, assessing, and correcting heuristic deficits.

Proposed Course of the Project: As a result of reduction in personnel two projects planned for the present reporting period have been deferred; the execution of a rule learning experiment and the development of an attribute identification simulator begun last year. Recent trends in the theory of human thought processes indicate that the completion of the simulator is essential to continued participation in the current activities of the scientific community in this area of distinct importance to mental health. An effort will, therefore, be made to reactivate this project. Because the study of rule learning is necessary to render the investigations of attribute identification logically complete, an effort will also be made to carry on that experiment.

Honors and Awards: None

Publications: None

Serial No. M-P-B-2
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Analysis of the relationship between problem-solving behavior and certain cortical and subcortical structures in the subhuman primate brain.

Previous Serial Number: Same.

Principal Investigators: H. Enger Rosvold, Mortimer Mishkin, Patricia Goldman

Other Investigators: Roger Buddington (NIMH Postdoctoral Fellow)

Cooperating Units: None

Man Years:
Total: 4
Professional: 1 2/5
Other: 2 3/5

Project Description:

Project: To identify the brain structures that are essential for problem-solving behavior.

Objectives: To determine the neural system or systems serving problem-solving behavior as exemplified in delayed-response, discrimination, learning set, etc., and to describe the nature of the behavioral deficits which follow damage to the structures comprising these systems.

Methods: (1) A variety of behavioral tests are used to define the functions which are specific to the prefrontal lobes as a whole, or to various of its parts. The current emphasis is to analyze the frontal-lobe deficit in terms of its participation in spatially directed responses and the utilization of interoceptive and exteroceptive cues. (2) Lesions are placed in subcortical structures (caudate nucleus, medialis dorsalis, hypothalamus) and the effects compared with those following selective frontal lesions. (3) Biochemical assay of tissue in frontal cortex and related subcortical structures.

Major Findings: The experiments described above (and others completed or nearly completed this year) were in progress at the time of the last annual report and the major findings are substantially the same: (1) Earlier work had suggested that the deficit in monkeys following dorsolateral prefrontal lesions is due to a disturbance in the utilization of response-produced cues elicited by spatial responses in a test. To assess this view, monkeys with dorsolateral prefrontal lesions and normal controls were trained to run to one side of a T-Maze under two conditions of illumination - dimly lit and well lit. When the maze is dimly lit, learning the spatial response should rely heavily on response-induced or proprioceptive cues and frontal monkeys should be impaired. In fact, the results did not confirm the prediction. While the data from "critical" trials interpolated between regular training trials indicated that the monkeys did indeed learn the task on the basis of proprioception, the frontal monkeys nevertheless performed as well, if not better, than the controls. In the well lit condition, by contrast, frontal monkeys were impaired and there was evidence to indicate that they attended to and were perhaps confused by the visual-spatial cues available in that situation. This study is the first of its kind to dissociate between sensory (response-produced or proprioceptive) and "gnostic" modes of spatial learning in frontal monkeys and to call into question the proprioceptive basis of the frontal disorder. (2) Earlier work had demonstrated that those functions which are differentially affected by dorsolateral and orbital cortical lesions are also differentially affected by lesions in subcortical structures which receive anatomical projections selectively from the dorsolateral or orbital frontal cortex. One of these subcortical structures is the medial dorsal thalamic nucleus. It has been a long-standing puzzle in neuropsychology that lesions in this nucleus do not result in "frontal" deficits despite the intimacy of its anatomical connections with the frontal cortex. Nevertheless, since the anatomical relationships are so compelling, we undertook to investigate this problem again with slightly different procedures. The results clearly indicate that lesions in this nucleus can result in profound deficits on these tasks. Current efforts are still being directed toward determining which of the factors introduced into the new study is critical in producing these deficits, and it now appears that lesion size is of paramount importance. Another of these subcortical structures having close anatomical relationships with the prefrontal cortex, is the hypothalamus. While many studies have demonstrated similarities between the frontal cortex and the hypothalamus in the emotional and motivational functions which they subserve, none has considered whether or not there are also similarities in cognitive functions. Our recent work, still in progress, has shown that large lesions involving different divisions of the hypothalamus result in a pattern of deficits on cognitive problems which are characteristic of damage to the orbital frontal cortex. Smaller, selectively placed lesions, though resulting in impairments, did not yield a pattern of deficits unequivocally characteristic of one or the other of the frontal-lobe systems. (3) See "Proposed Course of Project".

Scientific Significance to Mental Health: The results of this project have revealed that the prefrontal cortex in monkeys is functionally heterogeneous with dorsal and ventral areas participating in problem-solving behavior in two quite different ways; even within these areas there is evidence of further specialization of function. Furthermore, each of these frontal areas appears to be but one component of a larger cortical-subcortical system. Specification of the types of cortical contributions to problem-solving, and delineation of the two-cortical-subcortical systems, should help to resolve the longstanding puzzle regarding the functions of the frontal lobes.

Proposed Course of Project: While a great deal is known about the functions and anatomical connections of the prefrontal cortex, almost nothing is known about the neurochemical properties of this region. Do the anatomical pathways to and from the prefrontal cortex involve selective neurochemical transmitters? Can the major functional subdivisions of the prefrontal cortex be differentiated in terms of different neurochemical pathways? Does injury to the prefrontal cortex result in disturbance in metabolic functions in regions remote to the injury, for example the caudate nucleus or the hypothalamus? If so, are these effects slighter if the injury is sustained in infancy or in stages (e.g. serial lesion phenomenon). These and other questions are concerned with elucidating the relationship between the physiology, anatomy, and biochemistry of the neural substrates of behavior. Much is known about each of these properties of neural tissue separately but little attempt has been made to relate each to the other. This year the Section on Neuropsychology and the Laboratory of Clinical Sciences have begun to explore these questions collaboratively. An initial investigation is in progress on the effects of dorsolateral and orbital prefrontal lesions on the metabolism of biogenic amines in various regions of the central nervous system.

Honors and Awards: None

Publications:

Bowden, D. M., and McKinney, W. T.: Behavioral effect of peer separation, isolation, and reunion of adolescent male rhesus monkeys. Development. Psychhobiol. 5: 353-362, 1972.

Pohl, W.: Dissociation of spatial discrimination deficits following frontal and parietal lesions in monkeys. J. comp. physiol. Psychol. 82: 227-239, 1973.

Rosvold, H. E.: The frontal-lobe system: cortical-subcortical interrelationships. Acta Neurobiol. Exp. (Warzawa) 32: 439-460, 1972.

In Press:
Bowden, D. M. and McKinney, W. T.: Effects of selective frontal-lobe lesions on response separation in adolescent rhesus monkeys. Exp. Brain Res.

Marburg, D. L.: The effect on reaction to painful stimuli of lesions in the centromedian nucleus in the thalamus of the monkey. Intern. J. Neurosci.

Marburg, D. L.: The effect of lesions in the centromedian nucleus of the thalamus on the monkey's performance in delayed alternation and object reversal tasks. Intern. J. Neurosci.

Serial No. M-P-B-5
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neural mechanisms in vision.

Previous Serial Number: Same

Principal Investigator: Mortimer Mishkin

Other Investigators: Charlene Jarvis (NIMH Staff Fellow)
Jean Delacour (NIMH Guest Worder from College de France, Paris)
Lillian Blake (Consultant Part-time)

Cooperating Units: Charles Gross, Princeton University, Princeton, New Jersey
Frederick J. Manning, WRAIR, Washington, D. C.

Man Years:
Total: 5 4/5
Professional: 2 3/5
Other: 3 1/5

Project Description:

Project: Behavioral, anatomical, and electrophysiological analysis of neural mechanisms in vision.

Objectives: To define the role in vision of neural structures outside the primary visual projection system.

Methods: (1) Monkeys are prepared at NIMH with various lesions to the visual system, and they are then studied electrophysiologically at Princeton University by Gross and his colleagues to determine whether or not the receptive field properties of inferotemporal units have been altered in a manner predicted by our theory of cortico-cortical transmission. (2) Monkeys are tested after various inferotemporal and limbic lesions for visual short-term memory (delayed matching-from-sample) and visual associative learning (discrimination reversal) in an attempt to determine whether these two mnemonic functions can be dissociated, and selectively related to the anterior inferotemporal cortex and to the temporal pole and amygdala, respectively. (3) Unit activity in the inferotemporal and polar regions is recorded while a monkey performs a visual discrimination reversal to determine whether inferotemporal units might be sensitive to the sensory and not the affective quality (reward value) of a stimulus, and conversely, whether polar units might be sensitive to the affective and not the sensory quality.

Major findings: (1) In accord with predictions, left striate cortex ablation restricts the receptive zones of inferotemporal units in both hemispheres to the left visual field, while forebrain commissurotomy limits the units' receptive zones to the visual field contralateral to the hemisphere being recorded from. The latter effect appears from preliminary studies to be due primarily to transection of the spenium of the corpus callosum; transection of the anterior commissure by itself has no detectable consequence. (2) Preliminary data suggest that both the anterior inferotemporal cortex and the temporal pole and amygdala contribute to both types of mnemonic mechanisms (short-term and associative) though there may be relative differences between them; by contrast, the hippocampus appears to contribute to neither type of memory. (3) The behavioral and electrophysiological equipment for this study are being assembled.

Scientific significance to mental health research: Vision is perhaps the best understood of the sensory modalities in relation to the functioning of the nervous system. Yet a major problem remains unsolved in vision as it does in all sensory modalities: Delineation of the events which intervene between stimulus reception at the cortex and the observed response. The demonstration that damage to the inferior convexity of the temporal lobes in monkeys produces impairment in visually-guided behavior has opened up the hitherto inaccessible area of the intracerebral processes in vision, i.e., neural activity related to vision but beyond the level of the striate cortex. Unravelling these mechanisms in vision should aid greatly in the solution of a general problem for psychology, viz., accounting for the intervening neural processes (thought and its breakdown) in normal and abnormal behavior.

Proposed Course of Project: (1) Monkeys with selective transections of the splenium and anterior commissure are being added to the electrophysiological study in order to examine the reliability of the initial results. (2) Additional animals will be trained for each test-lesion group to increase the N sufficiently to permit statistical comparisons. (3) This study will be pursued through initial data gathering, at which time an evaluation will be made re continuing vs. modifying it.

Honors and Awards: None

Publications:

Jones, B. and Mishkin, M.: Limbic lesions and the problem of stimulus-reinforcement associations. Exp. Neurol., 36: 362-377, 1972.

Iversen, Susan D. and Mishkin, M.: Comparison between the effects of superior temporal and inferior frontal lesions on auditory and non-auditory tasks in rhesus monkeys. Brain Res. In Press.

Pohl, Walter: Dissociation of spatial discrimination deficits following frontal and parietal lesions in monkeys. J. comp. physiol. Psychol., 82: 227-239, 1972.

Serial No. M-P-B-7
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Histological analysis of cerebral lesions and intracerebral connections in primates.

Previous Serial Number: Same

Principal Investigator: H. E. Rosvold, M. Mishkin, Patricia S. Goldman

Other Investigators: T. N. Johnson (Consultant Part-time)

Cooperating Units: None

Man Years:
Total: 2 4/5
Professional: 4/5
Other: 2

Project Description:

Project: Verification of lesions in chronic experimental animals, and analysis of intracerebral connections in acute preparations.

Objectives: The objective of the first part of this project is to delineate the loci of the lesions which produce specific behavioral effects, and of the second, to determine whether or not loci yielding similar effects are anatomically interconnected.

Methods: When the behavioral testing of chronic experimental animals is completed, their brains are embedded in either celloidin (large specimens) or paraffin (smaller specimens), sectioned, stained for cells and fibers, examined microscopically, and the lesions reconstructed. In the second part of the project, animals are prepared with selective lesions purely for anatomical investigation. The animals are sacrificed within a few days after surgery and their brains examined microscopically to determine the locus, extent and course of degenerating fibers. Special staining procedures and fiber-size determinations are used to study stage of myelinization.

Major Findings: Specimens completely processed and examined include 110 monkey brains. This material has been examined for the purpose of verifying the loci of the cortical and subcortical lesions in animals used for behavioral studies.

In previous studies it had been demonstrated that there is a topographic projection of fibers from various parts of the prefrontal cortex to the head of the caudate nucleus and putamen. We have now completed a study demonstrating that this topographic arrangement is maintained in the efferent projections of the caudate to the globus pallidus, fibers from the dorsolateral caudate taking a lateral position in the pallidum, and fibers from the ventrolateral caudate taking a medial position in the pallidum. Current work is revealing that, in turn, the efferents from the lateral pallidum and from the medial pallidum are dissociable, each taking separate trajectories into the thalamus and subthalamus. However, while the projection of the lateral pallidum is rather selectively to the subthalamic nucleus, the projection of the medial thalamus is more disseminated through the thalamus and subthalamus. The reliability of these findings is being ascertained in additional preparations.

Scientific Significance to Mental Health Research: Sufficient evidence has been accumulated in behavioral studies with brain damaged animals to suggest that widely separated cell aggregates in cortex and subcortex may be combined into a complex system; and it is the functioning of such neural systems, rather than the functioning of the particular elements of which they are composed, that seems most directly relevant to an understanding of the organization and disorganization of behavior. The significance of this project lies in the fact that it can provide detailed information on the composition of these neural systems; systems which through electric and chemical manipulation have been related to mental health and disease.

Proposed Course of Project: The studies involving localization of function, anatomical substrate of the frontal systems, and anatomical substrate of the visual systems will continue.

Honors and Awards: None

Publications: None

Serial No. M-P-B-16
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cerebral mechanisms underlying functional plasticity in the developing organism.

Previous Serial No.: Same

Principal Investigator: Patricia S. Goldman

Other Investigators: H. Enger Rosvold
Epp Miller (NIMH Postdoctoral Fellow)

Cooperating Units: None

Man Years:
Total: 3 1/2
Professional: 1 2/5
Other: 1 4/5

Project Description:

Project: To determine the cerebral mechanisms underlying functional plasticity in the developing organism.

Objectives: Behavioral deficits following cortical injury are commonly less severe the earlier in life the injury is sustained. The major purpose of this research is to understand the mechanisms by which recovery is accomplished following brain damage in infancy and to determine whether or not similar mechanisms could be enhanced or induced when such damage is sustained later in life.

Methods: The studies involve the comparison of identical lesions in infants and juveniles. The infants are usually operated when they are 2-months of age; the juveniles between 1 1/2 and 2 years old. Both groups are allowed a 10-month postoperative recovery. Thus, the infants are 1-year of age and the juveniles 28-34 months old when formal testing of their cognitive abilities is begun. Unoperated monkeys of the same ages (that have also resided in the laboratory for ten preceding months) are tested as well. The studies have involved the following lesions: prefrontal lobectomies; dorsolateral prefrontal and orbital prefrontal resections; caudate nucleus lesions; combined prefrontal lobectomies and caudate lesions; and ablation of the dorsomedial nucleus of the thalamus. The behavioral tests utilized are designed to measure specific and dissociable functions of the prefrontal cortex.

The same tests are given in the same order to infants and juveniles alike. Upon completion of the testing, which may require a year or longer, the monkeys are sacrificed and their brains processed for histological examination.

Major Findings: The findings reported below are the same as those contained in the last annual report. The results of long-range studies now in progress cannot be anticipated (however, see section on Proposed Course of Research) but should be available in 1974. (1) Recovery of function is not an immutable consequence of early brain damage as the literature would imply. Lesions of the orbital cortex in infants, unlike those in other regions of the cortical mantle, produce impairments in behavior comparable to those produced by identical lesions in adults. (2) These impairments are equally severe whether the lesions are induced at 3 days, 1 week, 4 weeks, 8 weeks, or 130 weeks of age. (3) The capacity for recovery is directly related to the age of the animal at surgery but the relationship is almost the opposite of what might be expected. It might be supposed that the less mature the cortical area is when damaged, the greater the chance of recovery. Actually, if a lesion is made in an area such as the orbital cortex that develops early in ontogeny, there will ultimately be greater recovery than if a lesion is made in the dorsolateral cortex which develops later in ontogeny. We have proposed a theoretical frame work for interpreting these somewhat paradoxical phenomena, stressing the relative maturity of functionally related areas which are spared by the early injury. (4) Both the expression of deficits attributable to early brain damage as well as compensatory readjustments to such injury may be delayed until the brain approaches full maturity. Thus, monkeys given dorsolateral lesions in infancy appear to behave as well as normal controls at early stages in development but later in ontogeny there emerges a picture of retarded development. In the case of monkeys given orbital lesions in infancy, the adultlike deficits which are evident when testing is conducted in the first year and a half of life, tend to disappear when the monkeys reach two years of age. (5) Recovery mechanisms are not necessarily reciprocal. Our evidence suggests that the dorsolateral cortex may compensate for the loss of orbital cortex since monkeys given orbital lesions as infants recover at about the time the dorsolateral cortex approaches functional maturity. Monkeys given dorsolateral lesions, on the other hand, do not recover despite the integrity of the orbital cortex. (6) Subcortical functions may be more vulnerable to early injury than are cortical mechanisms. Lesions of the caudate nucleus and the dorsomedial nucleus of the thalamus in infants result in severe impairments irrespective of age at surgery. (7) Monkeys given caudate lesions as infants are impaired on just those tasks and at just those ages that the monkeys with dorsolateral lesions are not. This finding suggests that in the course of normal development, subcortical structures may be primarily responsible for mediating many of the functions that the cortex will ultimately assume.

Scientific Significance to Mental Health Research: By understanding mechanisms for recovery of function following neonatal injury, we may be in a better position to correct errors of normal development as well as approach the problem of recovery in the adult-brain injured.

Proposed Course of Research: Three studies bearing on the theoretical analysis of developmental plasticity include:

I. Long-Range Effects of Orbital and Dorsolateral Prefrontal Injury in Infancy. Monkeys operated upon in infancy (approximately two years ago) will soon be tested when they are two years of age. Behavioral assessment will extend until the monkeys are in the fourth year of life. This study will (1) replicate previous findings under more stringent conditions; (2) examine the role which experience during the first years of life may have had on the recovery or lack of recovery following orbital and dorsolateral prefrontal lesions, respectively; (3) extend longitudinally the period of observation so as to establish the permanence or reversibility of early brain damage; and (4) determine whether the subcortical mechanisms which appear to mediate behavior of early brain damaged monkeys at early stages of development can maintain this function or whether these mechanisms deteriorate with advancing age.

II. While the previous study extends the period of behavioral testing into the third and fourth years of life, another study employs the opposite strategy - that of constricting the testing period into the first year of life. Previous research has indicated that the orbital prefrontal cortex is functionally mature by one year of age. By comparing monkeys with orbital lesions and unoperated controls at 2 months, 6 months, and 9 months of age, if necessary, it should be possible to determine when within the first year, orbital function develops. Differences between operated and unoperated monkeys should not be evident at ages prior to the maturation of the orbital cortex. If the results of this study parallel those obtained in the case of monkeys with dorsolateral lesions tested at different ages, we will have demonstrated in two different neurobehavioral systems that deficits following early injury become evident only after the injured region would presumptively become functional. The different time course for each region would reflect regional differences in rates of development.

III. A third study is concerned with the specification of the neural substrates involved in behavioral recovery. Monkeys with orbital lesions in infancy are now growing up and will be tested for behavioral recovery at the two-year stage of development. At that time, those monkeys that demonstrate recovery will be given lesions in areas suspected of mediating the recovery, and then retested. The results of this study will bear on the theoretical analysis of plasticity in developing monkeys and provide evidence about what CNS structures are involved.

IV. Neuroembryological and neurochemical studies have been initiated this year with the aim of elucidating the biological limits of plasticity. Monkeys will be given prefrontal lesions during gestation, replaced in utero, and if carried to term, delivered by cesarian section. Behavioral studies will be conducted over the first few years of life. The neuroembryological experiments constitute a critical test of specific theoretical issues regarding plasticity but it is hoped that such inquiry will open the way for primate neuroembryological studies in general. So far, one monkey given a dorsolateral lesion in the second trimester of gestation has been successfully delivered. He is in excellent condition. This work is being conducted in collaboration with investigators at Bionetics, Rockville, Maryland, and the Institute for Behavioral Research, Silver Spring, Maryland. The neurochemical studies are described elsewhere in this report as they deal primarily with adult monkeys. We consider that these studies in mature animals will serve also as a basis for the analyses of developmental questions. Finally, correlative neuroanatomical studies of the developing monkey are underway. These are described in a separate section of the annual report.

Honors and Awards: None

Publications:

Goldman, Patricia S. Developmental determinants of cortical plasticity. Acta Neurobiol. Exp. (Warszawa) 32: 366-387, 1972.

Goldman, Patricia S. and Rosvold, H. E. The effects of selective caudate lesions in infant and juvenile rhesus monkeys. Brain Res. 43: 53-56, 1972.

In Press

Goldman, Patricia S. Recovery of function after CNS lesions in infant monkeys. In, Functional Recovery After Lesions of the Nervous System. Eidelberg, E. and Stein, D. G. (Eds.) Neurosciences Res. Prog. Bull.

Serial No. M-P-B-17
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neuroanatomical basis of function development; and investigations of structural correlates of behavioral recovery following early brain damage.

Principal Investigators: Patricia S. Goldman, H. E. Rosvold

Other Investigators: T. N. Johnson (Consultant, Part-time)

Cooperating Units: None

Man Years:
Total: 1 3/5
Professional: 3/5
Other: 2 3/5

Project Description:

Project: Neuroanatomical development.

Objectives: (1) To describe the development of cortical-subcortical connections in normal ontogeny; (2) To search for evidence of new anatomical pathways resulting from early brain injury that would be capable of mediating behavioral recovery.

Methods: (1) Monkeys are given unilateral orbital or dorsolateral prefrontal lesions at 2 months, 6 months and 24 months of age. Fourteen days following surgery, the monkeys are sacrificed and their brains processed by the technique of silver impregnation, as well as by conventional methods of cell staining. Frontal sections through these brains are examined microscopically for evidence of anterograde and retrograde degeneration. (2) Monkeys that received bilateral orbital prefrontal lesions as infants and have demonstrated recovery of function at two years of age will be given unilateral lesions in regions of the brain hypothesized to be involved in mediating the recovery. These areas include the dorsolateral prefrontal cortex, the premotor cortex, the medial frontal cortex, the anterior temporal lobe and the amygdala. Controls are monkeys that receive unilateral lesions of the above areas at the two-year stage of development, without having had bilateral orbital surgery in infancy. Some of the preparations in (1) can serve as controls in (2). Following the unilateral surgery at two-years, the monkeys are sacrificed and their brains processed as in (1).

Major Findings: Approximately 12 monkeys have been prepared for (1). More monkeys are needed to examine parameters which affect anterograde degeneration differentially in young and adult brains. Microscopic analysis of some of the brains is beginning and the results are not available at this time. Ten monkeys have been given bilateral orbital lesions during the past year but will not be ready for anatomical surgery for 1-2 years.

Scientific Significance to Mental Health: The brain is the organ of normal and abnormal behavior. Neuroanatomical studies together with behavioral, electrophsyiological, and neurochemical approaches that shed light on how the brain develops provide a basis for understanding errors of development. Developmental anatomical studies may increase our understanding of the limits of central nervous system plasticity not only with respect to recovery from traumatic injury but with respect to experiential factors which may speed or retard development.

Proposed Course of Research: New project as described.

Honors and Awards: None

Publications: None

Serial No. M-P-L-5
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Individual Differences in Normal Perceptual Processes

Previous Serial Number: Same

Principal Investigator: V. R. Carlson

Other Investigators: Irwin Feinberg

Cooperating Units: VA Hospital, San Francisco

Man Years

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: General: (1) To obtain normal control data for standardizing procedures and apparatus in the measurement of perceptual variables, and to obtain comparison data for evaluating the effects of abnormal and other special conditions. (2) To develop a general specification of perceptual response in terms of the parameters of the immediate stimulus-situation, past experience, generalized perceptual-cognitive attitudes, and the subject's motivational reaction to the situation.

Changes in time sense are reported to accompany THC intoxication. It is not clear, however, whether subjective attitude about time or the capacity to discriminate the passage of time is altered. The purpose of the present study is to determine whether THC affects verbal judgments of duration or the nonverbal functional use of time.

Methods Employed: In order to assess verbal judgments of time, subjects are asked to estimate the durations of presented time intervals varying from 1 to 60 secs. For nonverbal functional use of time, subjects perform a perceptual-motor task in which a moving spot of light traverses a portion of a screen and disappears. The subject operates a key when he judges that the moving spot would reach a designated target location beyond the point of disappearance of the spot. Both kinds of data are obtained under placebo, low dose, and high dose drug conditions on different days.

Major Findings: Preliminary investigation has been done to work out the details of the testing procedures, and data collection is currently in progress.

Scientific Significance to Mental Health Research Program: Temporal disorientation is involved in many abnormal psychological states. Whether it represents a contributing cause or a result of other aspects of disordered behavior is not known.

Proposed Course of Project: If the results of this study warrant, an attempt will be made to develop a more generally applicable approach to the problem of differentiating effects on verbal attitudes about time from effects on the capacity to discriminate time accurately.

Honors and Awards: None

Publications: None

Serial No. M-P-L-7
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Perceptual Adaptation

Previous Serial Number: Same

Principal Investigator: V. R. Carlson

Other Investigators: Eugene Tassone, Harry Blum, Francine Frome

Cooperating Units: DCRT, University of Maryland

Man Years

Total:	2.0
Professional:	1.0
Others:	1.0

Project Description:

Objectives: General: To investigate the characteristics of perceptual adaptation, both when the adaptation occurs in a single limited period of persistent stimulation and when it is cumulative in a series of stimulations over relatively long periods of time.

The occurrence of visual adaptation to stimulus motion, form, and orientation suggests the operation of specialized receptive units of the kind which have been identified in cat and monkey visual cortex. Such adaptation is also basically similar, however, to a conditioning or learning process. The purpose of one experiment was to determine whether adaptation to motion dissipates over a period of neutral stimulation, as would be expected if the process is one of sensory adaptation. A second experiment was concerned with the applicability of a new theory of biological form generation to the perception of the orientation of a visual configuration. The theory postulates a field-like process not restricted to the local activity of receptive cells stimulated by the contours of a figure.

Methods Employed: In the motion experiment subjects were exposed to a rotating pattern for 15 sec. Then, after exposure to a homogeneous gray surface for 0-8 sec., they judged the apparent speed of a single rotating line. In the form orientation experiment subjects matched the orientation of a series of ellipses and elliptical parallels to the orientation of a straight line. The figures were constructed to provide variation both in conventional geometric properties and in the symmetric axis, a parameter hypothesized to be the perceptually important property.

Major Findings: Prior adaptation to the moving pattern altered subsequent perception of the speed of the test line, but the effect was not diminished by introducing a delay between adaptation and test. This result is more characteristic of a memory process than a strictly sensory one. In the form experiment orientation judgments varied with variation in the symmetric axis when the major diameter or focal axis was constant. The judgments did not vary with variation in the major diameter or focal axis, when the symmetric axis was constant. This result indicates that the symmetric axis represented the relevant information for perception of the orientation of the figures.

Scientific Significance to Mental Health Research Program: Specialized receptive neurons sensitive to particular directions of motion and orientation of contours probably exist in the human brain and are necessary for the perception of motion, form, and orientation. But the results imply that such cells are not sufficient to account for the basic perceptual phenomena involved. An understanding of how perception develops will most probably have to be concerned with conditioning and learning processes as well as physiological maturation.

Proposed Course of Project: The extent to which perceptual adaptation occurs to specific combinations of stimulus variables, such as motion and form or motion and color requires elucidation. The value of a concept such as the symmetric axis lies in its derivation from a general theory, and its usefulness in different kinds of stimulus situation needs to be explored.

Honors and Awards: None

Publications: None

Serial No. M-P-L-9
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Discriminative and Conceptual Behavior in Preschool Children

Previous Serial Number: Same

Principal Investigator: Albert J. Caron

Other Investigators: None

Cooperating Units: Private and Cooperative Nursery Schools in Montgomery County and the District of Columbia

Man Years: None

This project was temporarily suspended and is being held in abeyance.

Honors and Awards: None

Publications: None

Serial No. M-P-L-10
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Discriminative and Conceptual Behavior in Infancy

Previous Serial Number: Same

Principal Investigator: Albert J. Caron

Other Investigators: Rose F. Caron

Cooperating Unit: Eye Research Foundation

Man Years:

Total:	2.25
Professional:	1.50
Others:	.75

Project Description:

Objectives: To assess the perceptual-cognitive capacities of human infants during the first year of life and to investigate the antecedent environmental conditions promoting early perceptual and intellective development.

Study I: The specific aim of a series of experiments was to determine which features (eyes, nose/mouth, head, inner features) predominate in the four- and five-month-old's perception of the human face and to what extent these are seen as isolated elements or as structured configurations. A more general objective was to assess the applicability of Eleanor Gibson's differentiation theory to the development of object perception in early infancy. According to this view, the ability to discriminate objects develops through the gradual extraction of their distinctive features, beginning with the most salient properties and ending with more obscure elements and patterns.

Methods Employed: Fixation time to an intact schematic face was compared for different groups of infants (7 boys and 7 girls per group) following prolonged exposure to a distorted version of the schematic face, under the assumption that the greater the discriminability between the familiarized distortion and the intact face, the longer would be the looking time to the latter. The groups differed only in terms of the particular distortion presented for familiarization. These included aberrations of four areas--eyes, nose/mouth, head, and inner face--and were of four general types: (a) elimination of feature, (b) displacement, (c) reorientation, and (d) scrambling. A control group was familiarized with the intact face.

Major Findings: The results of an initial experiment indicated that at four months of age the eyes are more salient than the mouth, and are also seen as a clearly defined structure (paired in the horizontal axis and located in the upper half of the head). In addition, the head, including hair and face contour, was found to be more prominent than the inner face configuration. In a second experiment, it was demonstrated that the responding of the most extreme groups in Experiment 1 had indeed been under discriminative control and was not an artifact of the particular stimuli used in these conditions. The findings of a third experiment revealed that at five months of age the mouth has become as distinctive as the eyes, and the inner face configuration as salient as the head. Overall, the data tend to support Gibson's position.

Study II: The purpose of research now in progress is to determine whether the three- to four-month-old infant has achieved perceptual shape constancy, i.e., whether objects retain their shape despite shifts in perspective.

Methods Employed: We had initially attempted to conduct this experiment using a discriminative operant conditioning procedure, but have since discovered that our discriminative stimuli are being presented at a distance which probably exceeds the range of the young infant's visual acuity (a limitation of our apparatus). We are currently overhauling this apparatus, but in the meantime have constructed equipment which will allow us to carry out a parallel experiment using a familiarization-novelty technique. Subjects will be exposed to a frontal rectangle (or frontal trapezoid), appearing in each of two adjacent target windows for 2 minutes. Following familiarization, one of the stimuli will be changed either to a backward tilted rectangle (which projects retinally as a trapezoid) or to a forward tilted trapezoid (which projects retinally as a rectangle). To the extent that the infant discriminates the novel and familiarized stimuli, he will look longer at the novel one and thus provide an indication of whether he perceives retinally or objectively.

Major Findings: As mentioned, most of the work thus far has been in the pilot stages. With the discriminative operant technique we were successful in conditioning babies, but not in bringing responding under discriminative control. We are currently piloting the familiarization procedure and the results appear promising.

Study III: The purpose of a third project is to determine whether differential responding and/or differential consequences are necessary for infants to learn to discriminate between stimuli (or whether they can learn by simple exposure to stimuli and without the occurrence of correlated consequences).

Methods Employed: Groups of three-month-olds will receive discriminative operant training over a four week period using stimuli which had previously been determined to be non-discriminable by the average four-month-old. One group will be required to make differential responses (e.g., head turn vs. no head turn) to two stimuli (e.g., circle and triangle) which in turn lead to differential rewards (visual and auditory events vs. no consequence). A second group will not be required to emit differential responses but will receive visual and auditory events in the presence of the circle and no such event in the presence of the triangle. A third group (a simple exposure group) will not be required to emit differential responses nor will it experience differential consequences in the presence of the circle and triangle. Appropriate control groups to assess the independent contributions of responding, reinforcement, and operant training will be employed. Apparatus has just been completed which will allow for automatic presentation and recording of these various types of contingencies.

Major Findings: Four-month-olds are currently being tested by means of familiarization-novelty procedure in order to select stimuli which are non-discriminable at this age, and hence which may be used in the discriminative training phase. Training has not yet been undertaken.

Scientific Significance to Mental Health Research Program: The three studies, taken together, will give us some insight into the young infant's perceptual capabilities and, what is more important, into the environmental arrangements which helped foster these attainments. Once we have learned how the environment impresses itself on the maturing organism to produce a stable perceptual world, then we are in a position to manipulate the sensory input to infants to promote optimal perceptual and intellective development.

Proposed Course of Project: This project is an indefinitely continuing one in pursuance of the general objectives stated above.

Honors and Awards: Elected Fellow, American Psychological Association (Div.7).

Publications:

Caron, A. J., Caron, R. F., Caldwell, R. C., & Weiss, S. J.: Infant perception of the structural properties of the face. *Developmental Psychology*, 1973, in press.

Serial No. M-P-L-12
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cortical Mechanisms in Somethesis

Previous Serial Number: M-P-B-12

Principal Investigator: Josephine Semmes

Other Investigators: None

Cooperating Units: None

Man Years
Total: 3
Professional: 2
Other: 1

Project Description:

Objectives: The general aim is to identify in monkeys the neural mechanisms concerned in various froms of somesthetic perception, and more specifically, to differentiate functionally the respective contributions of the lemniscal and extra-lemniscal somatosensory cortical projection areas. Recent experimentation has focussed on the sensorimotor cortex contralateral to the hand trained on a series of manual discrimination tasks and of acquired motor responses. The immediate purpose is to define the behavioral significance of the various subdivisions of this general region. Behavioral tests are necessary in order to confirm, deny, or modify hypotheses derived from electrophysiological and anatomical studies concerning the functional organization of the region and its relation to the connections (afferent and efferent) of each subdivision.

Methods: Rhesus monkeys are trained to make somesthetic discriminations requiring them to reach with one hand into an opaque box and select one of two stimulus objects, indicating their choice by pulling it toward them. For each pair, one of the objects (randomly interchanged in position) is consistently rewarded whereas the other is not. Discriminations tested in this situation have involved consistency, shape at three levels of difficulty, size, roughness, and temperature, and for the last three, repeated determinations of difference limens have been made. A passive situation has also been used: the monkey is restrained in an opaque box with the hand to be tested (outside his vision) extending through an opening in the box and held flat on a platform by straps. An instrumental response, pushing open a hinged door to obtain the reward on positive trials, is performed with his free hand.

For example, the degree of sensitivity to light touch, as measured by a series of graded nylon filaments, is determined in this manner. Motor tests range from simple measures of strength and dexterity, through gestures toward an external object or toward part of the monkey's own body, to complex sequences made up of different instrumental movements performed in a prescribed order.

Ablations of the selected cortical areas are made by gentle aspiration restricted to grey matter, performed when necessary under a magnifying lens. Aseptic technique, barbiturate anesthesia, and antibiotics (topical and systemic) are routinely employed. The postoperative recovery period is usually four weeks (though it has been extended to six months or more in certain studies), after which training or retraining is begun. Following completion of the behavioral observations and formal tests, the animals are given a lethal dose of barbiturate, and their brains are prepared for histological examination. The actual limits (and depth) or the cortical lesion, as well as the consequent degeneration of parts of the thalamus, are then determined. Special attention is given to unintended sparing of tissue within the limits of the planned ablation and to unintended damage to grey or white matter outside these limits.

Major Findings: Research in this unit has established that performance on the motor tests at all levels of complexity are impaired by lesions in front of the central sulcus (precentral gyrus and premotor area), but that such lesions leave the sensory discrimination tests entirely unaffected. Lesions behind the central sulcus do, however, affect performance on the motor tests, although the deleterious effect is relatively slight. Our further work has focussed on the sensory/perceptual effects of lesions behind the central sulcus, with the aim of confirming and extending our previous findings. We have confirmed that ablating the anterior part of Somatosensory area I (S I), the postcentral gyrus, produces extremely severe sensory deficits on most tests, but that touch and temperature thresholds are not affected; we have recently also found that size thresholds are consistently much less affected than discriminations involving consistency, roughness, or shape. These deficits are not ameliorated by extending the recovery period in the home cage to 6 or 7 months. Extensions of the lesion to include the entire postcentral gyrus (instead of only the contralateral hand representation), or removing the hand representation from both hemispheres (instead of one) seem to have a slight additional adverse effect (rather than a paradoxical ameliorative one, as we thought possible). Within the contralateral anterior postcentral gyrus, the part forming the wall of the central sulcus is by far the most important behaviorally, a fact consistent with its receiving the densest lemniscal projection (shown anatomically by others). The posterior part of the postcentral gyrus, and the still more posterior region of the parietal extent, in other types. We have undertaken a study of five groups of monkeys, with progressively more posterior small lesions, in the attempt to find whether or not a functional boundary between S I (the principal lemniscal area) and the posterior parietal lobule (an extra-lemniscal area) can be established. Definitive results cannot be reported as yet, since the inter-individual variability of the subjects makes it necessary to add more animals to each group. We are also studying unilateral and bilateral lesions of Somatosensory area II (S II), the parietal operculum, an area which can be considered mainly, although not exclusively, lemniscal. This area, which has a complete somatopic representation separate from that of S I, has been functionally mysterious, since others have found no deficits following its removal. With our battery of tests, we have been able to demonstrate impairments (a distinct advance), but we have not yet arrived at a proper characterization of these deficits in comparison to those of S I, nor have we been able to establish whether or not bilateral lesions of S II are consistently more deleterious than are unilateral. Again, more animals in each of the two lesion groups should help in resolving the issue, but perhaps new tests will have to be added also.

Scientific Significance to Bio-Medical Research: Our results, it is hoped, will clarify the respective roles in behavior of the lemniscal and extra-lemniscal afferent pathways - pathways which have markedly different electrophysiological properties. Tentatively, we are now of the opinion that active touch and active movement (as opposed to passive) are primarily related to and guided by the lemniscal system, but that refined sensation (tactual or kinesthetic) is possible through the extra-lemniscal pathway, if the stimulation does not require exploratory movements or manipulation of an active sort. Because of the high degree of connectivity between the lemniscal sensory and the pyramidal motor systems, we are also entertaining the hypothesis that, in the case of motor cortex or pyramidal tract lesions, training in sensation may aid in recovery from motor defects, resulting either from experimental or naturally-occurring lesions. Conversely, sensory defects should be lessened by training in systematic movements. The wealth of the somatic afferent input to the cortex, and of the efferent outflow from it (extra-pyramidal as well as pyramidal), suggests that the prognosis, with respect either to sensory or motor impairment, is more hopeful than has been fully recognized in actual practice so far.

Future Course of Project: The most immediate information needed is clarification of the posterior postcentral gyrus-posterior parietal contribution(s) to somatosensory discrimination and a more precise formulation of the role of the parietal operculum. Beyond these advances in knowledge on the purely sensory/perceptual side, the interrelations between sensory and motor functions need to be more carefully explored, not only from the point of view of recovery from one or the other kind of defect, but also from the perspective of how the sensory and motor systems cooperate to make possible normal sensation and movement.

Honors and Awards:

1. Membership, Experimental Psychology Study Section, DRG, 1969-1973
2. Editorship, *Cortex*, 1968-

Publications:

Semmes, J. and Porter, L.: A comparison of precentral and postcentral cortical lesions on somatosensory discrimination in the monkey. *Cortex*. 8: 249-264, 1972.

Semmes, J., Porter, L., and Randolph, M.C.: Further studies of anterior postcentral lesions in the monkey. Cortex, in press, 1973.

Semmes, J. Somesthetic effects of damage to the central nervous system. In Jung, R. (Ed.): Handbook of Sensory Physiology. New York, Springer-Verlag, in press (to be published in 1973).

Serial No. M-S-C-11
1. Socio-environmental Studies
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Social Psychological Correlates of Occupational Position

Previous Serial Number: Same

Principal Investigators: Melvin L. Kohn and Carmi Schooler

Other Investigators: None

Cooperating Units: None

Person Years

Total:	3 1/4
Professional:	1 1/4
Other:	2

Project Description:

Objectives: To ascertain the relationship of some principal dimensions of job, occupation, and career to relevant aspects of men's values, social orientation, self-conception, and off-the-job behavior.

Methods Employed: Structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations in the United States, conducted in the spring of 1964.

Major Findings: Our major finding this year is that the major finding presented in last year's annual report was right--for the wrong reasons. We concluded then, and can affirm now, that the relationships between occupational conditions and psychological functioning result from a continuing interplay between job and man, in which the effects of job on man are far from trivial.

In last year's report, we presented systematic evidence demonstrating that occupational conditions, particularly those we call the structural imperatives of the job, are significantly and substantially correlated with many facets of psychological functioning, ranging from self-esteem to intellectual flexibility. We then examined a number of possible interpretations of these correlations, presenting several strands of evidence, none definitive but all consistent in indicating that the relationships between the conditions men experience in their work and their psychological functioning do not simply reflect some inadequacy in our data; or the effects of education, occupational status, and income; or men's efforts to mold their jobs to fit their needs and values; or selectivity in recruitment and retention. These negative arguments were necessary, in order to establish the plausibility of an interpretive model that assumes reciprocal effects of job on man and man on job. This having been established, the last and crucial step was to assess the magnitude of the reciprocal effects. Here is where we failed last year, and think we have succeeded this year.

Our former approach was to stretch conventional statistical procedures (namely, multiple regression and path analysis), which had been designed to measure the magnitude of effect in situations where one-way directionality could be assumed, to fit the analysis of two-way directionality. In retrospect, we tried to do the impossible; conventional statistical procedures simply cannot be used to assess reciprocal effects. But we have since learned that econometricians have developed a number of simultaneous equations techniques for dealing with precisely this problem, techniques that are well known and well validated in econometrics but little known and heretofore almost entirely unused in the other social sciences.

In our analysis, we use the technique called two-stage least squares, a relatively simple method for estimating coefficients in simultaneous equations. The two-stage procedure, in effect, attempts to "purge" each variable of the effects of all others with which it is reciprocally related, by estimating from other pertinent data what each individual's score on that variable would have been if the other variables had not had an opportunity to affect it. These estimated scores are then used for assessing each variable's impact on the others.

As prototypic analysis, we examine the reciprocal effects of the substantive complexity of the job and intellectual flexibility. Substantive complexity is chosen for this analysis because it is the single occupational condition having the most potent psychological ramifications, and also the only one for which we have job-history information fully adequate to the demands of this type of analysis. Intellectual flexibility is chosen because it appears to offer us the toughest test: Intellectual flexibility is obviously pertinent to recruitment into substantively complex jobs, and it might be expected to be the most resistant to change of all facets of psychological functioning we have studied.

The analysis shows that the effects of job complexity and intellectual flexibility are, as the interpretive model assumes, reciprocal: each is a major determinant of the other. The model also enables us to assess the relative magnitudes of effect of substantive complexity on intellectual flexibility and of intellectual flexibility on substantive complexity. The ratio is exactly two to one. In terms of their present reciprocal relationship, the impact of job on intellectual flexibility is greater than the reverse.

It is a simple extension of the interpretive model to consider the relationships between substantive complexity and several other facets of psychological functioning we have measured in this research. As before, the crucial comparison is of the effect of the substantive complexity of the job on a particular facet of psychological functioning, as compared to the effect of that facet of psychological functioning on substantive complexity. In all ten comparisons that we are able to make, job affects man more than man affects job. Thus, the substantive complexity of the job is consistently important for psychological functioning, and consistently more so than is psychological functioning for substantive complexity.

All this is based on but one dimension of occupation, its substantive complexity. We cannot say whether it is true for the job as a whole, as it is for substantive complexity, that the impact of job on man is consistently greater than the reverse. But we can conclude that in the continuing interplay between man and job, the effects of job on man are substantial.

Scientific Significance: In industrial society, occupational role is of central importance in shaping men's conceptions of self and of social reality. This research is addressed to a systematic appraisal of precisely which aspects of occupation affect which facets of man's social psychological functioning.

Proposed Course of Project: There are a number of other important problems that can be pursued with the data in hand, e.g., the social psychological effects of occupational mobility. But the central problem, measuring and interpreting the effects of the job on the man, requires a follow-up study of a subsample of the men in the 1964 survey. Even the application of econometric techniques to the analysis of the reciprocal effects of job on man and man on job cannot definitively show change in particular individuals. Nor does it provide much information about the learning mechanisms involved in transferring the lessons of the job to nonoccupational realms of life. We need to re-study a substantial subgroup of our original sample, recording all changes in their occupational conditions since the original survey in 1964, and re-measuring all the principal facets of psychological functioning examined in the earlier study. We are presently trying to arrange the financing for such a study.

Honors and Awards: Melvin L. Kohn: elected to membership in the Sociological Research Association; appointed to the Board of Directors of the Foundations' Fund for Research in Psychiatry

Publications:

Kohn, M. L.: Class, family, and schizophrenia: a reformulation. Social Forces, 50: 295-304, 1972.

Schooler, C.: Childhood family structure and adult characteristics. Sociometry, 35: 255-269, 1972.

Schooler, C.: Social antecedents of adult psychological functioning. American Journal of Sociology, 78: 299-322, 1972.

Schooler, C.: Birth order effects: not here, not now! Psychological Bulletin, 78: 161-175, 1972.

Kohn, M. L. and Schooler, C.: Occupational experience and psychological functioning: An assessment of reciprocal effects, American Sociological Review, 38: 97-118, 1973.

Schooler, C.: Birth order effects: a reply to Breland. Psychological Bulletin, in press.

Serial No. M-S-C-14
1. Socio-environmental Studies
2. Office of the Chief
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Social Adaptations and Mobility of Urban Black Poverty Families

Previous Serial Number: None

Principle Investigator: Albert J. McQueen

Other Investigators: None

Cooperating Units: None

Person Years

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives: The main objective of this research is to formulate a theoretical model that reveals the essential social processes and structural contingencies embracing the efforts of black families to cope with urban poverty. The two broad dimensions of family adaptive problems dealt with involve severe resource deprivation and access, based on the societal structure of power for allocating resources and highly devalued social status in situations where there are few institutional supports for maintaining or enhancing family status positions.

Four family types have been identified: the coping poor, the marginal poor, the coping near-poor, and the marginal near-poor. These types denote styles of relative effectiveness in utilizing financial resources for family needs, the coping types being most effective and the marginal ones least effective. The focus of my efforts is to understand the social and attitudinal bases predisposing parents to choose different coping strategies in the consumption and disposition of resourses.

Methods Employed: Two related research undertakings are involved. An earlier attitudinal survey was made of mothers in 100 poor black families in the Cardoza area of Washington, D. C. At present a series of case studies of 15 to 20 poor black families in the same area is being carried out by myself and graduate students at Howard University. We are using intensive interviewing and participant observation methods to gain a more comprehensive picture of the range of coping patterns and consequences.

Major Findings: Black parents have devised a variety of ingenious consumption and social practices to insure survival and in quest of viable family life under imposed conditions of frequent hunger, recurring crises, and persisting insecurity and uncertainty. Adapting to urban life, uneven employment opportunities, low and often unsteady income, dangerous environments, and other such trying conditions often builds great strength and perseverance in black parents. At the same time, however, such conditions permeate their lives with continuing crises and failures, or at least omnipresent threat of them. Despite their strong family values and often courageous efforts to realize family aspirations and goals, many black parents are pushed into a state of despair and hopelessness and are unable to fend off destructive ghetto forces of compressed misery and exploitation constantly pressing upon their families.

Despite the myriad barriers of racism and limited opportunity, many poor parents do hold tenaciously onto their social mobility values and manage somehow to raise their standard of living, however little, and their sense of worth and respectability. They often must do this at the high cost of sacrificing certain present consumption and comfort needs in order to convert a portion of their resources into developmental capital for the future. Although many such families are not able to move from the slums, they are geared to creating conditions and opportunities that increase the chances that the children in them will move up in society. These type families I have characterized as incipiently socially mobile ones.

Scientific Significance: This adaptation approach to the analysis of poverty families displaces the more simplistic notion of culture of poverty that is commonly indiscriminantly used to explain the nature and generational persistence of poverty. Not only does it yield a more realistic picture of the richness and diversity of family life in poverty, but it also holds the promise of providing powerful analytical tools for the scientific study of families cross-culturally and in contrasting socio-economic circumstances. Importantly, this approach can be instrumental in dispelling value-laden myths and stereotypes about black families that have taken root in social science.

Proposed Course of Project: I am now writing a book on the earlier study. It will be completed during the next three or four months. The data-gathering for the family case studies should be completed by the end of May and analysis and writing undertaken during the summer.

Honors and Awards: None

Publications: None

Serial No. M-S-D-10
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Research on the Processes of Internalization of Rules, Standards, and Values

Previous Serial Number: Same

Principal Investigator: Roger V. Burton

Other Investigators: None

Cooperating Units: None

Person Years

Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: Work this year under this project has been primarily directed toward two goals. The first is an extensive review of previous research on moral conduct, and the second is the planning of a new program of experimental studies in moral conduct.

1. Reserarch Review. An invited review of research in moral conduct is being prepared for Man and Morality: Theory, Research, and Social Issues, edited by Thomas Lickona, to be published by Holt, Rinehart, and Winston. Assessment is made of the empirical research based on the behavioral, cognitive-judgmental, and affective components of moral conduct. The issues covered in the review are the generality-specificity of honesty both within and across the three major components, the generality of functional relations found for situational determinants of honesty--including age, intelligence, sex, norms, modeling, risk, incentives, fear of failure and need for success, and other personality characteristics--socialization effects on honest conduct, and a theoretical integration of the findings in a social learning framework.

In addition to the usual stocktaking purpose of this review, the broad perspective and critical assessment of the previous work has served as a basis for planning a new program of experimental studies that are now being formulated and readied for pretesting.

2. Experimental Studies in Training for Generality of Moral Conduct. Previous studies (Hartshorne and May, 1928; Burton, 1963) have shown that moral conduct is primarily situationally determined although there is evidence of a low order of generality across many different test situations. The generality of personality constructs are usually assessed by analyzing the correlations across the various tests of that construct.

An alternative approach is to group individuals by the consistency of their behavior in order to explore what personality and background variables relate to differing degrees of integration. Analyses again are correlational, providing theoretical leads but only tentative evidence for causal explanations.

A social learning model has been suggested for integrating and explaining currently available evidence on the generality-specificity of honesty (Burton, 1963). This model proposes that behavior transfers from situation to situation as a function of two independent, generalization gradients. The first gradient is a physical similarity dimension, the same dimension of stimulus similarity studied in conditioning and animal learning experiments. The second gradient is cognitive and similar to the dimension studied in concept acquisition experiments. The model proposes that these generalization gradients combine to determine differences in consistency of behavior. Predictions can be derived from the model about the relation of certain child rearing and training techniques that would contribute to increasing the consistency in an individual's honest conduct. The studies now being developed will explore these hypotheses about rearing techniques directly, using experimental designs. In brief, these studies will experimentally vary certain factors in modifying behavior in specific situations and subsequently assess the effects of these treatments across other diverse tests of honesty. The current objective in this program is to develop a basic procedure (experimental paradigm) which will reliably demonstrate that the various treatments produce differential degrees of consistency in moral conduct. Once this procedure is developed, factors that might affect the additive function of the two generalization gradients will be systematically explored.

Methods Employed: Measures. Tests used for training or shaping behavior must permit individual administration and the possibility of rewarding and punishing behavior in the test itself while the subject performs alone. A bean bag game, already developed, serves as the prototype of this kind of test situation. Scores for this game appear to be gained by hitting a string that trips a microswitch. Each "hit" rings a bell and turns on a light. In fact, the game is completely controlled by a hidden experimenter. This type of control allows the hidden experimenter to reward (or punish) the subject for playing honestly (or dishonestly) when by himself.

The main variables for this initial paradigm will be (1) the degree of apparent similarity across honesty tests and (2) the specificity-generality of the critical verbal labels used in training the child how to play. A series of honesty tests will be chosen and modified, or developed if necessary, for the present purposes. The tests now being considered vary in both manner (e.g. cheating in games vs. cheating in tests) and type (cheating, lying, stealing) of deception involved. Temptation tests being explored are games with rules, attractive but tabooed toys and candy, turning in "lost" money, peeping tests, and telling the truth about one's role in an "accident." In addition to their being ranked for similarity on logical grounds (e.g. games with rules, situations involving delay of gratification, etc.), a similarity gradient will be empirically established by the magnitudes of the intercorrelations among the tests.

Treatments. The cognitive structure provided the child will vary along differential degrees of generality in the critical labels used in the training. In the low cognitive structure condition, the child is told only "Do it like this," or "No, No! Don't do that." It is hypothesized that this highly specific kind of prohibition will have little effect on the child's behavior in other temptation situations. Variations toward increasing generality of cognitive structure will be to label correct behavior with broader concepts that would be more inclusive across the different tests. Examples (though not completely developed yet) are (1) "You should do this because it is a rule in this game"; (2) "1" plus ". . . because it would cheating if you didn't follow the rules of a game"; (3) "2" plus ". . . cheating is not being honest. You wouldn't be honest if you cheated by breaking the rule." Additional variations will be to have the child give examples of other situations that would fit the critical label being used at each level of conceptual generality. For examples: (1) "Do you know another game with rules that you should follow?"; (2) "What other kinds of cheating do you know about?"; (3) "Besides following the rules of games, do you know other ways of being honest or what things some people do that might be dishonest?"

Initially the subjects will be 6-7 year old children. Children of this age seem appropriate for working out the basic procedure as they are acquainted with the critical terms, and yet their understanding of the concepts is still likely to be limited and vague. Learning what is included by these critical labels is probably linked with general cognitive development as measured in moral judgment studies and would be an extension of what Piaget has studied as class inclusion. Findings from this research lead us to expect that children of this age should be quite specific in their behavior initially and are likely to show most clearly the effects of the training conditions on the acquisition of the concept related to overt behavior.

Consistency of behavior is more likely in older children initially. Also research in verbal mediation indicates that older children would learn the implication of the critical concepts faster and demonstrate broader and more lasting effects of the training procedures. However, there may be additional performance (as distinct from acquisition) effects that will have to be considered with these other children. Therefore, before expanding the age of the subjects, the experimental procedure will be developed with the younger children who are closer to a developmental age in which these concepts are being acquired.

If successful in developing experimental paradigms that demonstrate reliable effects in shaping consistency of behavior, we shall also extend the studies to explore what processes might be determining the effects of the treatments. For example, is it simply a cumulative effect of instating inhibition or is there really a hierarchical arrangement of critical labels? The effect of the status of the rule establisher can be studied by using parents and peers as instructors. Using parents as instructors would further permit us to assess the parents' natural (i.e. uninstructed) use of the different treatments and to observe whether this "natural" use produces effects that parallel the experimental paradigm. It has been suggested that interactions with peers determine the development of moral judgment (Piaget, 1932, Kohlberg, 1964). It may also be that in the learning of moral conduct peers are more influential than adults, especially as models for establishing normative behavior. Comparisons of peers, parents, and adult strangers would shed important theoretical data on the contribution these different agents make to the acquisition of individual standards of honesty. These extensions suggest the potential directions of this program when a stable procedure is established.

Major Findings: Data are not yet available.

Scientific Significance: These studies will contribute to an understanding of socialization process, particularly to the inculcation of moral standards involved during temptation to deviate from established norms for moral conduct. This information will add important empirical data necessary to the building of a comprehensive theory of socialization and of the development of conscience.

Proposed Course of Project: Pretesting will begin and lead to the major data gathering stage for this experiment. From these data, scientific papers will be prepared for publication.

Honors and Awards: None

Publications:

Burton, R.V.: The interpretation of intergenerational attitudes. Merrill-Palmer Quarterly of Behavior and Development 18: 61-62, 1972.

Serial No. M-S-D-26
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: An Observational Study of Maternal Models

Previous Serial Number: Same

Principal Investigators: Carolyn Zahn Waxler and Marian Radke Yarrow

Other Investigators: None

Cooperating Units: National Institute of Child Health and Human Development

Person Years

Total:	1/12
Professional:	1/12
Other:	0

Project Description:

Objectives: To examine interactions between mothers and their 20-month-old children in relation to patterns of maternal behavior that influence imitative responding, and in relation to theoretical formulations regarding the origins of imitation. In particular, to examine sequences of behavior in terms of a reinforcement theory of generalized imitation, and to identify characteristics of parental models associated with imitative responding in their children. This study extends investigation to a much younger age than that of the usual subjects in experimental work on imitation.

Methods Employed: Thirty-five black mothers from middle and low economic classes were observed with their children in a planned series of settings which permitted natural and semi-natural interaction. Mother and child were observed in a laboratory trailer during a brief waiting period, in play, in teaching situations, and in a planned modeling session. Later, an experimenter repeated the mother's modeling. On another occasion, the children were given the Bayley Developmental Test. Interactions were observed, with the mothers' knowledge, from behind a one-way screen. Observer reliabilities on exact matching of sequential behavior units range from 68% to 87%.

Major Findings: Findings have been reported in the previous annual report. Where those findings led to further questions, new analyses have been undertaken, namely: Analyses are now in progress concerning characteristics of mother's reinforcements, other than schedules or frequency, to ascertain relations to children's imitative behaviors. Further, characterizations of the imitative behaviors and examination of interaction of child and mother characteristics are being undertaken.

Scientific Significance: Observed interactions of mothers and children under field conditions can (a) test principles formulated from laboratory research, (b) raise questions about these formulations, and (c) provide new evidence that will further understanding of the processes involved.

Proposed Course of Project: Very little staff time has been available for this project this year. Analyses will continue during the coming year.

Honors and Awards: None

Publications: None

Serial No. M-S-D-28
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Comparison of Methods of Obtaining Data on Parent and Child Behavior

Previous Serial Number: Same

Principal Investigators: Marian Radke Yarrow and Carolyn Zahn Waxler

Other Investigators: Thomas A. Padrick

Cooperating Units: None

Person Years

Total:	1/12
Professional:	1/12
Other:	0

Project Description:

Objectives: To compare information concerning child rearing and child behavior obtained by different and accepted methods of assessment, and to examine particular problems associated with different data-gathering procedures.

Methods Employed: Sixty preschool children and sixty families were the subjects. From an interview with the mother, summary assessments of her rearing practices and her child's behavior were obtained. In the following two weeks, there were four hours of observations in the home. Observations were in the form of sequential accounts of mother and child behavior. Ratings of mother and child behavior in the home were also made, based on the same periods of observation. At the close of the second session in the home, the mother was interviewed about the behavior that had occurred during that session.

Behaviors were assessed in the following dimensions: mother's warmth, coldness, demonstrativeness, rewarding, and punishing of the child's dependency; child's compliance or dependency, "conscience," and aggression. In coding the interviews and in making the observational ratings, the same categories and scales were used. In coding the observations, the indicators for each variable were as similar as possible to those used in the interviews. The observational records were time-marked in 30-second intervals. Based on the proportion of time units in which the variable occurred, subjects were assigned decile scores on each variable.

The interviewer and the observer of a given subject were never the same person. All interviews were coded independently by two coders; intercoder reliabilities ranged from r's of .51 to .93. Dual observations carried out in the nursery school were the basis of an estimate of observer reliability in the home. Correlations ranged from 4's of .50 to .89. Variable observer agreement on different behavioral dimensions was one of the interests in the analysis.

Analyses are directed to examining the congruence of assessments (a) of the same variable across methods, and (b) of relationships between variables--both within the same method and across methods. There are eight maternal variables and six child variables in four within-method comparisons, and in six across-methods comparisons.

Associations between variables across data sets and within data sets were analyzed using Pearson correlations and Kendall's Tau coefficients.

Major Findings: Findings have been reported in the previous Annual Report.

Scientific Significance: Preceding work in this program of research has been concerned with methods of investigating child and parent behaviors through the use of verbal reports. In those studies, major and distressing inconsistencies were frequently found in the empirical data deriving from different measuring techniques. The present project extends the investigation to methods using direct observations. An appraisal of observational data, along with verbal report information, is important to the understanding of the methods upon which research and theory rely.

Proposed Course of Project: Little staff time has been given to this project in the past year. Methods developed in it are being used in projects 29 and 30. Organization of the data from this study will continue toward the preparation of a scientific article.

Honors and Awards: None

Publications: None

Serial No. M-S-D-29
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Development of Sensitivity to Others' Feelings

Previous Serial Number: None

Principal Investigators: Marian Radke Yarrow, Carolyn Zahn Waxler, Robert A. King

Other Investigators: Elizabeth N. Conklin, C. Jean Darby, Marilyn T. Oliver, Thomas A. Padrick, Frances L. Polen

Cooperating Units: None

Person Years

Total:	2 5/6
Professional:	1 2/3
Other:	1 1/6

Project Description:

Objectives: Little is currently known about the growth and early development of the young child's ability to perceive other people's feelings, motives, or points of view, and the nature of his responses to them. A project is planned to study, longitudinally, the development of such abilities. This study focuses on the second year of life. Equal numbers of boys and girls--10 infants, beginning ages 9 to 10 months; 10 infants, beginning ages 15 to 16 months; 10 infants, beginning ages 21 to 22 months--will be the subjects.

Methods Employed: The primary data to be obtained are (1) the child's responses to the feelings of others (persons, animals, dolls, etc.) (a) when the feelings are relatively independent of the infant's own activities and needs, and (b) when the infant is a participant in the situation evoking feelings in the other person; and (2) the child's hurting, sharing, giving, nurturing, showing affection to others.

Additional data will be obtained concerning (3) parents' responding to the feeling-states of the child, and (4) the child's engagement in social interactions that appear to indicate estimates by the child of the other person's feelings and characteristics.

The sources of data will be the mothers' observations and reports (following the model provided by the classic study of children's anger by Goodenough, 1931). The mothers will also be "experimenters." They will arrange a number of semi-standard situations. These are to be created at the mothers' convenience in as natural a context as possible.

At three-week intervals, the investigator(s) will visit the home (a) to obtain data on the child's reactions in semi-standard situations in which the expression of feelings is involved; (b) for discussion of mother's observations of the preceding three weeks; and (c) for systematic observations of child and mother-child interactions. At less frequent intervals, simultaneous observations by mother and investigator of child during some portion of the visit will be used to assess observer reliability.

The analysis framework will include a normative maturational analysis; i.e., a delineation of regularities in patterns of development. In the developmental descriptive analysis one will look for qualitative changes in signs of recognition of others' feelings, and in kinds of affective responses and other-directed responses elicited in the child.

Accompanying data on the child's imitation, play, and communication will provide an additional frame of analysis in which one can examine possible covariance with behaviors.

Relations will be examined between the child's responses and the mother's handling of both the child's feelings and the affect in the child's social environment.

Major Findings: None. The project is in the early stages of data gathering.

Scientific Significance: The lack of systematic evidence on early manifestations of this aspect of the child's communication with other persons leaves developmental psychology with only armchair theories in a very vital area of personality development. The science is without a normative base regarding the child's abilities to comprehend the attributes or states of others. We are without a clear picture of the early indicators of normal or pathological development. Psychopathology often involves significant distortions or limitations in the individual's awareness of other people's feelings and his sense of similarity or difference from others (e.g., childhood autism). Our understanding of many forms of psychopathology would be advanced by a more thorough knowledge of the early phases of the normal child's evolving sensitivity and responsivity to the feeling states of others.

Proposed Course of Project: Data collection and analysis will continue for the next two years.

Honors and Awards: None

Publications: None

Serial No. M-S-D-30
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Developmental Studies of Altruism

Previous Serial Number: None

Principal Investigators: Marian Radke Yarrow and Carolyn Zahn Waxler

Other Investigators: Elizabeth N. Conklin, C. Jean Darby, Robert A. King, Marilyn T. Oliver, Thomas A. Padrick, and Frances L. Polen

Cooperating Units: None

Person Years

Total:	3 1/3
Professional:	1 1/6
Other:	2 1/6

Objectives: This research is focused on altruism, developmentally considered--children's potentialities for altruism and the ways in which compassionate behaviors are socialized.

1. What are the developmentally changing cognitive abilities of the child to recognize and take the perspective of others with regard to their affective states; and what are the parallels between these abilities and the perceptual-cognitive abilities in the more literal sense involving the physical world?

2. How does the ability to take the "feelings" perspective or role of the other relate to the child's responding altruistically?

3. What are the socialization influences in developing and maintaining a strong altruistic component in the child's personality or behavior repertoire?

Methods Employed: Sample: Approximately 100 children, ages 3 to 5 years of age, in attendance at a nursery school.

Design A: The child's sense of the perspective or role of the other person is examined at three levels: (a) perceptual-cognitive role taking, (b) social-emotional perspectives, and (c) overt responding to the social-emotional distress of another person. A series of experimental situations is administered in the forms of games and problem-solving tasks and projects. In the perceptual-cognitive realm, the child's ability to put himself in the place of O in a visual, perceptual sense (situations from Piaget and Flavell) is measured. Standard situations involving social-emotional perspectives in general parallel the perceptual tasks in formal properties. The inferences required by the child are concerning feelings of O. Standard situations will test children's sharing, helping, and sympathy. The situations represent varied distresses, varied intensity of cues, and varied "causes" of the victim's distress, and increasing degrees of difficulty.

Design B: Training will be undertaken to increase sensitivity and responsiveness to others' needs, building on the effective experimental condition of the past study (Learning Concern for Others). The training will incorporate a number of "knowns" about modeling and reinforcement effects and will adapt and elaborate on them to increase the likelihood of self-regulation by the child. Training procedures include:

(a) Sensitization to cues in others by coupling experiences involving others' feelings with "cognitive enrichment" (explanation of feelings) from the adult.

(b) Reinforcement of responses of help, sharing, sympathy, in terms of the consequences of S's action for the recipient.

(c) Modeling of sensitivity to feeling cues and of altruistic response, using naturally occurring and introduced events.

A second phase of experimentation involving mothers is planned. Mothers will be selected and randomly assigned to one of three conditions: (a) a control group; (b) a group in which mothers are engaged in reporting on their child's empathy and altruism or insensitivity and indifference; and (c) a group in which mothers will be guided in carrying out training similar to the training carried out in the nursery school.

Major Findings: Project is in the pilot stage.

Scientific Significance: Although surely a valued dimension of human experience, altruism has not seriously engaged the interest of either theorists or investigators in developmental psychology. Investigation of the individual's humane capabilities has not kept pace with research interests in areas such as achievement motivation, competitive striving, aggression, power assertion, anxiety, and guilt. Research on altruism needs to be pursued on many fronts--for scientific and humanitarian reasons, for the fact remains that investigation of the idealized behaviors of kindness and sympathy toward fellow beings has moved slowly in the behavioral sciences. Moreover, there is no compelling evidence to indicate that, in the rearing of its children, society has given high priority to the inculcation of personal concern for the welfare of others.

Proposed Course of Project: Data collection and analysis will continue for the next two years.

Honors and Awards: None

Publications:

Yarrow, M. R., Scott, P. M., and Waxler, C. Z.: Learning concern for others. Developmental Psychology, in press.

Serial No. M-S-P(C)-23
1. Socio-environmental Studies
2. Personality and Environment
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cultural and Psychodynamic Factors in the Occurrence and Treatment of psychiatric Illnesses in Japan, Taiwan, and the United States

Previous Serial Number: Same

Principal Investigator: Carmi Schooler

Other Investigators: Hsien Rin

Cooperating Units: Department of Psychiatry, National Taiwan University, Taipei, Taiwan

Person Years

Total:	1 1/4
Professional:	1/4
Other:	1

Project Description:

Objectives: (1) To identify and describe the types and symptom patterns of psychiatric illnesses in Japan and Taiwan. (2) To determine the relationship of these types of illnesses to social, cultural, and psychological variables in Japanese and Chinese cultures. (3) To compare the results obtained with results from similar data on psychiatric patients in the United States.

Methods Employed: Symptom data discussed in the present annual report were gathered by having doctors in each hospital in Japan and Taiwan fill out a research instrument for each patient which obtained relevant background and family information and a semi-structured narrative description of the course of the patient's illness. The narrative description was then coded for the presence or absence of 90 symptoms; the set of symptoms represents an extended version of the symptom checklist used in an earlier study. All symptoms which occur in more than 7 1/2% of the combined Japanese and Taiwan Chinese population are included in a series of principal components factor analyses that are rotated to simple structure. The subjects in the two samples consist of: (1) all admissions to five psychiatric hospitals in Tokyo during a twelve-month period in 1963-64 for a total of 994 patients; (2) all admissions to three psychiatric hospitals in Taipei during a two-year period, 1967-68, for a total of 1,009 patients.

Major Findings: Three major findings have emerged from the comparison of Japanese and Taiwanese symptomatology. The first is that factor analysis of the symptom patterns of hospitalized Taiwan Chinese and Japanese mental patients reveals both cross-culturally applicable and culturally-specific symptom factors. The second major finding is the power and clarity of the cross-cultural differences in psychopathology which emerge. While the Japanese are likely to have disorders in arousal levels, to turn against themselves, and to see the major source of their problems as being within themselves, the Taiwan Chinese are much more likely to direct their symptomatology outward, to act out against others, and to perceive the outside world in unreal ways. The third major finding is that women in each culture are more likely than men to both show more severe symptomatology and to have a higher incidence of the symptom patterns which reflect the particular symptomatology of their culture.

A comparison of the symptomatology of Japanese public hospital schizophrenics in 1958 and 1964 revealed a significant reduction in the prevalence of 5 of the 7 symptoms which had distinguished the symptom pattern of the earlier patient group from a sample of similar American schizophrenics hospitalized between 1941 and 1949. On the other hand, the other two symptoms distinguishing Japanese symptomatology in 1958 had increased in significance by 1964.

Scientific Significance: The general tenor of the above findings is consonant with the hypothesis that the change in symptomatology found between our 1958 and 1964 Japanese samples reflects the growing similarity of socio-economic conditions in the United States and Japan. Furthermore, since the economic development of Taiwan may be about 15 years behind that of Japan, it is conceivable that, with the passage of time its pattern of symptomatology would also come somewhat closer to that of the United States in the late 40's and Japan in the mid-sixties. The six-year period from 1958 to 1964, during which the Japanese became significantly more affluent and more modern marked the decline of outwardly directed physical hostility among schizophrenics and the emergence of a general symptom pattern in which the root of one's difficulties is seen as being within oneself (i.e., shinkeishitsu, depression, somatization). This latter trend also marked the symptomatology of those in Japan and Taiwan who had achieved a relatively high social class. These findings of a relationship between inwardness in the direction of the symptomatology and modernization or upward mobility are quite congruent with earlier social psychological findings with normal individuals. Evidence exists that both the childhood exposure to a relatively complex multifaceted environment and the experience of self-direction on the job, which is more likely to occur among those at the higher occupational levels, are likely to have the direct effect of making an individual focus increasingly on his inner life.

However, even if we are correct in assuming that our data provide some evidence of a relationship between socioeconomic development and symptomatology, our data also contain plentiful evidence that historically determined cultural factors also have their residual effects. Thus, between 1958 and 1964 the Japanese showed an increase in the amount of sleep disturbance, a symptom pattern which may well be related to culturally determined sleeping habits.

In an earlier study, Caudill concluded "I believe that in this paper sufficient evidence has been presented to indicate that modern social structure and historical culture should be treated as separate variables, and that both have fairly independent effects on behavior and psychological adjustment. As countries develop a modern social structure in line with the requirements of increasing industrialization and technological advancement, then they do thereby become more alike; but at the same time this does not mean that the traditional culture is lost, rather it persists in many important ways that deeply influence a person's way of thinking, emotional response, and behavior." On the whole, Caudill's conclusions are equally applicable to the results of the present study. However, as is usually the case in social science, a complication has been added. Caudill's belief that socio-economic and cultural phenomena operate independently of each other must be amended. Our findings indicate that these two classes of variables not only have independent effects but can and do interact. Furthermore, since several of our major findings may be explained by either, the relative explanatory power of these two determinants of adult psychological behavior remains an open question.

Proposed Course of Project: The above findings have been written up and submitted for publication. Future analysis will center on the comparison of Taiwanese and American mental hospital patients for both of whom we have data based on the Lorr Inpatient Multiphasic Psychiatric Scale.

Honors and Awards: None

Publications: None

Serial No. M-S-P(C)-27
1. Socio-environmental Studies
2. Personality and Environment
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Parental Care and Child Behavior in Japan and the United States

Previous Serial Number: Same

Principal Investigator: Carmi Schooler

Other Investigators: None

Cooperating Units: None

Person Years

Total:	1 1/4
Professional:	1/4
Other:	1

Project Description:

Objectives: (1) To identify and describe patterns of parental care and child behavior in Japanese and American families. (2) To determine the relationship of these patterns of behavior to personality development, psychopathology, culture, and social structure in the two countries.

Methods Employed: The basic design of the study and methods used have been described in detail in previous reports. Briefly, this is a longitudinal observational study of the behavior of parents and children in the home over the first six years of life in Japan and the United States. During 1961-64, 30 Japanese and 30 American middle-class mother-infant pairs were selected for observation. The infants were first-born, normal babies, and were three-to-four months of age at the time of observation. The first 20 Japanese and the first 20 American cases were selected for follow-up study at 2-1/2 years of age, and observations in the home were completed during 1963-1967. These same children were studied for a third and final time at 6 years of age during 1967-70, and all observations have been completed.

Major Findings: This year has been spent in the completion of the coding of the data from the 2-1/2 and 6-year old children and preparing the data for computer analysis. We have also started to systematically examine the data in three ways: (1) we have looked for cultural differences in the behavior of both parents and children at the 2-1/2 and 6-year levels. (2) We have looked for consistency of behavior within individuals across time; e.g., from infancy to 6 years of age. (3) We have made preliminary attempts to examine relationships between parents' childrearing practices in infancy and behavior of the child at ages 2-1/2 and 6. In the latter analysis the data from each culture is being analyzed separately in the hope of being able to show that similar childrearing practices have similar effects in both cultures. Such a conclusion is necessary if one is to ascribe the cross-cultural differences in children's behavior to differences in childrearing patterns.

At this time the results are very preliminary. In general, there is an indication that the greater amount of physical activity and verbal behavior that was earlier reported as characterizing the American mothers and their children at the infant stage continues to distinguish the two cultures at the later observation points. Some indication also exists that these characteristics are moderately stable within individuals. On the other hand, up to this point in time we have not been able to definitively link parental childrearing patterns in infancy with later child behavior. In sum, at present the general tenor of the present findings suggests that American children live in a socially and physically more complex and multifaceted environment at each of the three ages we studied and that they react to this complexity by showing both a greater amount and a greater variety of verbal and physical activity.

Scientific Significance: The general purpose of this study is to show how historically determined cultural differences between societies can affect the behavior patterns of individuals. Furthermore, by having longitudinal data on the same individuals we should be able to not only examine the degree of consistency over time in a child's behavior, but also to gain some idea of which points in time, if any, are pivotal for his development.

Proposed Course of Project: Analyses will be continued and the results prepared for publication in a scientific journal.

Honors and Awards: None

Publications:

Caudill, W.: Tiny dramas: vocal communication between mother and infant in Japanese and American families. In Lebra, W. P. (Ed.): Mental Health Research in Asia and the Pacific, Volume Two. Honolulu, East West Center Press, in press.

Caudill, W.: A comparison of maternal care and infant behavior in Japanese-American, American, and Japanese families. In Lebra, W. P. (Ed.): Mental Health Research in Asia and the Pacific, Volume Three. Honolulu, East-West Center Press, in press.

Serial No. M-S-P(C)-38
1. Socio-environmental Studies
2. Personality and Environment
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Interrelationships between Social Interaction, Psychological Functions, Perceptual Style, Physiological Arousal and Personal History Factors Among Schizophrenics

Previous Serial Number: Same

Principal Investigator: Carmi Schooler

Other Investigators: Monte S. Buchsbaum

Cooperating Units: Laboratory of Psychology, NIMH; Saint Elizabeths Hospital; DCRT

Person Years

Total:	1 1/4
Professional:	1/4
Other:	1

Project Description:

Objectives: The objective of this series of studies remains the attempt to explain the variance found in earlier experiments with schizophrenics by discovering the interrelationships among those variables which these earlier studies indicate are central to an understanding of schizophrenia: task performance, physiological arousal, perceptual style, social behavior, symptomatology and psychiatric history.

Methods Employed: Several approaches are presently being followed. (1) The level of physiological arousal and task performance under different social conditions of both schizophrenics and nonschizophrenics have been measured and related to variations in perceptual style and personal history. (2) A major study has been initiated which involves the interrelating of measures of evoked response, perceptual style, skin temperature (a measure of physiological arousal) and symptomatology. (3) In order to facilitate the continuance of both of the above types of study, an attempt is being made to develop a generalized computer program to simplify the analysis of measure of physiological arousal and to relate these measures to each other and to other aspects of the experimental situations.

Major Findings: This year, just as last year, has been devoted primarily to the collection of data both at Saint Elizabeths Hospital and at the National Institutes of Health for the above-mentioned study of the interrelationships between evoked response, perceptual style, skin temperature, and symptomatology, and to the continued development of the general computer program to analyze physiological measures of arousal.

Scientific Significance: Since clinicians had long noted that social withdrawal appeared to be a marked feature of schizophrenia, and experiments by Schooler and others had demonstrated that the greater the degree of social contact the greater the decrement in the schizophrenic's functioning, it seemed reasonable to initiate a series of studies seeking the key to the schizophrenic's malfunctioning in his reactions to his social environment. In one of these studies, Schooler and his co-workers were able to establish through direct observation of ward behavior that lack of social interaction is, indeed, one of the characteristics which differentiates schizophrenics from other hospitalized mental patients. Furthermore, the results of another study indicate that this lack of interaction is mirrored by an expressed preference for avoiding entanglements with others. Additional studies in the series have shown how the schizophrenic's performance in standard psychological experiments is affected by orientations which are at least partially the results of his past social experiences. Thus, one study indicates that the effect of positive or negative evaluation on verbal-paired associate learning is a function of the subject's self-esteem; subjects with low self-esteem doing especially poorly on tasks requiring novel associations after being given positive evaluations of their performance which are dissonant with their negative self-image.

Several other studies point to the possibility that society's general norms as to sex roles have differentially affected male and female schizophrenics' willingness to show or receive signs of positive or negative feelings. Their findings are congruent with the supposition that in our culture the expression of emotion, and particularly of affection, is an area of conflict for men, while for women a major area of concern is fear of evoking the hostility of others. The possibility that social psychological factors can even affect psychophysiological relationships is indicated by the findings of another study which show that the social nature of the situation can affect the direction of the relationship between physiological arousal and task performance.

The interaction between the social situation, the schizophrenic's functioning, and his physiological reactivity pointed to a general weakness in the way this series of studies had been carried out. It had been conducted primarily from one research perspective and was concerned with only a limited area of the individual's functioning. Although the findings were significant they had not been very powerful, possibly because differences in the nature of physiological reactivity of different subgroups of schizophrenics may have produced contrasting, or at least differing, results.

A review of the literature revealed that the general concentration on one aspect of the individual which had marked these social psychologically oriented studies was at least as pronounced in studies in other areas. It was therefore not at all surprising that the weak results which marked the series of social psychological studies were at least as powerful as the results of studies approaching the problem of schizophrenia from other directions. Thus, for example, years of search for biochemical differences between schizophrenics and others have resulted in no reliably replicable findings. The effects of genetics appear to be less and less powerful, the better controlled the study. Putative social class differences between schizophrenics and others do not seem to exist under all conditions and where they do exist it is far from clear whether such differences are related to the cause of the disease or its effects. Those theoretically based systems of classifying patients into subgroups which have been investigated have not produced consistent findings and, in fact, the most highly touted mode of classification--pre-morbid adjustment--proved totally unable to pass the test of controlled replication.

The lack of findings has certainly not been due to any lack of theories or hypotheses. Hypotheses have been brought forth with the ease of the proverbial blind men describing the elephant, e.g., those studying tails describing the elephant as long, thin, and floppy; those studying problem-solving seeing the essence of schizophrenia in thought disorder.

Two courses seem to be presently open. One can keep on making hypotheses from limited bodies of data, hoping to achieve the flash of insight which will permit us to see from our limited point of view what the elephant is really like, or one can go ahead with the duller task of trying to do studies which attempt to coordinate data from many vantage points in order to get enough information to make meaningful hypotheses possible. The present series of studies represents an attempt to follow the second of these courses with the aid of modern multivariate techniques and computer technology. It thus involves the interrelation of five different types of variables which seem to be the most promising in terms of their possible etiological significance, their consistency in predicting schizophrenic responses, and their amenability to experimental manipulation. These five classes of data are (1) perceptual functioning, (2) central and autonomic nervous system arousal, (3) task performance, (4) social interaction, (5) psychiatric symptomatology and history.

Studies were undertaken which involved the replication and evaluation of earlier studies of schizophrenia in these five areas in a way which would permit the examination of their interrelationships among different schizophrenic subgroups. These studies of the investigator and his collaborators are now starting to show that many of the previously contradictory findings in the area of schizophrenia research have been due to an incomplete comprehension of the complexity of the interrelationships between psychiatric symptomatology, patterns of physiological arousal, and perceptual and social functioning among different types of patients.

Proposed Course of Project: Data collection will be continued and analyses of already collected data will be continued and the results prepared for publication in a scientific journal.

Honors and Awards: None

Publications: None

Serial No. M-S-PS-1
1. Socio-environmental Studies
2. Personality and Stress
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Health Orientations of Parents and Children

Previous Serial Number: Same

Principal Investigator: John D. Campbell

Other Investigators: Iris Posner

Cooperating Units: Children's Hospital, Washington, D. C.

Person Years

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives: To study health orientations of family members. Specifically, the goals of the study are, first, to describe systematically the patterns of variation in family members' attitudes, values, beliefs, perceptions, and behavior concerning phenomena of illness and health; and second, to examine ways in which selected personal and situational factors relate to health orientations. Relevant to the attainment of these objectives are the following three assumptions basic to the study: (a) an individual's orientation to health and illness is embedded in interactive processes; (b) such orientations are at least in part learned; and (c) to a considerable extent such learning takes place as an aspect of routine family relationships.

Methods Employed: Information on 264 hospitalized children and their mothers was obtained by use of focused interviews, questionnaires, and rating scales. Interviews with children and mothers, and questionnaires completed by the mothers provide data on (a) aspects of their definitions of illness, (b) reports of their own and other family members' feelings and actions when ill, (c) their attitudes and beliefs about illness prevention and treatment, (d) their values in selected areas not directly relevant to health, and (e) views about appropriate standards of behavior: what should or should not be done in circumstances pertaining to illness and health. Ratings, made by nurses in the hospital setting, appraise children's emotional dependence and anxiety.

Major Findings: Central to this research is the question of how health orientations are acquired, how modified. A commonly held view for this and other areas of social development is that parental perspectives, mediated by certain intervening variables, are transmitted to and adopted by the child. Evidence summarized in a previous annual report cast some doubt on the tenability of this view for at least one aspect of health orientations: comparison of mothers' and children's definitions of illness showed no systematic link between a child's concept of illness and that of his mother, at either the level of specific thematic content or a more abstract level of qualitative appraisal of sophistication of definition. This apparent lack of correspondence has led to examination of another area of health orientations, that concerned with normative judgments and standards, to see whether here, too, parental transmission was absent. Answers from identical questions to mothers and children enabled comparisons on the following dimensions: (a) standards of permissible health-relevant risk taking, (b) standards for open expression of emotion in illness and treatment, (c) standards for communication of feelings, (d) standards concerning desired degree of similarity between sick-role and normal role behavior, and (e) attribution of responsibility to the child for his own illness.

These normative areas are clearly more value-laden, more sensitive, than the cognitively neutral one of illness definition. Thus it might be reasonable to expect that a mother would be concerned about transmitting and would in fact be more likely to communicate her own perspective to her child. When mothers' normative appraisals were correlated with those of their children, there was evidence of a general positive relationship, although the degree of association between maternal and child norms was not profound. Measurable correspondence was the rule: if a mother held her child to be responsible for illness, her child was more apt to adopt that view; the child who thought one should express little strong emotion in illness tended to have a mother with a similar perspective, etc. Yet it would be more appropriate to describe the child's normative judgments as generally modestly paralleling his mother's, rather than equaling them. Though correlational analysis showed similarity, it was generally the case that children's and mothers' standards were not equally strict. (For example, children's views on appropriate health-relevant risk taking, though correlated with those of their mothers, were more relaxed than those of the parent.)

Correspondence between mother and child in these areas was slightly greater for the older, rather than the younger child. This suggests that the older child is more fully aware of and has adapted to the normative standards of his parents. But inculcation of standards is one thing; performance may be another: The relation between mothers' standards for their child and children's reports of their own functioning reveals an interesting pattern that can be illustrated by data concerning health-relevant risk taking. The association between mothers' standards and children's reports of risk-taking behavior was highly significant: children of mothers whose standards were high (i.e., mothers who disapproved of such risk taking) described themselves as not taking chances. Further, older children's reports of their risk taking corresponded more closely with their mother's standards than was the case for younger children. But importantly this does not mean that older children's reported behavior was an undistorted reflection of maternal standards. Despite the greater correlation for mothers and older children, the latter were significantly more likely than the younger to describe their behavior in a fashion that fell short of their mothers' expressed standards. The older child has learned the norms of his parent, has taken them as his own, but in so doing has also taken them with a grain of salt. That is, he apparently makes allowances for his mother's standards, but systematically acts in a measurably "riskier" fashion than his mother would sanction--a discrepancy more pronounced for the older children than for the younger.

Scientific Significance: This research provides information about a specific area that is currently only inadequately covered; it should lead to a more systematic picture of health orientations and factors associated with such orientations than is now available. The potential value of the research is not, however, restricted solely to the confines of sickness and health. Within the framework of the study, such general social psychological issues as role learning, developmental changes in family relationships, and role behavior in stressful conditions can be treated. The phenomena of illness and health can be viewed as representing specific aspects of these general issues.

Proposed Course of Project: Data analysis continues. One paper has been submitted for publication. A second paper, concerned with factors associated with children's interpreting certain signs and symptoms as indicating illness, is approaching completion. Other research reports will be written after appropriate data analysis.

Honors and Awards: None

Publications: None

Serial No. M-S-S-12
1. Socio-environmental Studies
2. Section on Social Structure
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Developmental Study of the Self-Image

Previous Serial Number: Same

Principal Investigator: Morris Rosenberg

Other Investigator: Roberta G. Simmons (University of Minnesota)

Cooperating Units: None

Person Years

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: To learn how children's self-images change as they grow older and to gain increased understanding of the social factors that influence self-image formation. While most previous systematic research has focused on self-esteem, the present investigation considers a number of other aspects of the self-image as well: degree of self-consciousness; preoccupation with the self; the quality of self-detachment; stability of self-concept; "front" or "presenting self"; committed and idealized images; development of ego-extensions; and other aspects of the self-image. The aim is to understand how these aspects of the self-image develop; to examine their relationships to a range of social conditions and experiences; and to learn how they are associated with other psychological states, such as anxiety, vulnerability, and depression.

Methods Employed: Structured and open-ended interviews. A stratified random sample of 1910 children from the third through the twelfth grades in a large urban school system was interviewed. Additional brief interviews were conducted with 400 parents to obtain certain essential background information.

Major Findings: (1) Self-image development--Among the most widely held assumptions in the behavioral sciences is the view that adolescence is a period of disturbance for the child's self-image (Hall; Erikson; Davis). Yet Offer's longitudinal study of 14-18 year old boys suggests that these years are not characterized by stress or turmoil. Other investigators (e.g., Grinker; Elkin and Westley; Douvan and Adelson) also question the assumption of adolescent crisis. Aside from psychiatric case histories, in fact, there is little evidence to refute or support the position that the child's self-image changes from childhood to adolescence.

The present study was designed to shed light on this issue. Since the age range of our respondents is mostly between 9-18, it is possible to compare various aspects of the self-image among different age groupings, even though longitudinal data are lacking.

The following questions were posed: (1) Do systematic data support the impressionistic evidence that the adolescent self-image differs from that of younger children? If so, is this difference one that can reasonably be characterized as "disturbance," i.e., an experience of discomfort or unhappiness? (2) If there is an adolescent crisis, when does it begin? Adolescence covers a span of many years. Does the disturbance appear in early, middle, or later adolescence? (3) If adolescent self-image disturbance appears at a certain point in time, what is the course of its further development? Do the problems continue to grow, level off, or decline? (4) If there is self-image disturbance, what triggers it? The precipitating factor is usually assumed to be biological. Are there also aspects of the social environment which may help to set off such a disturbance?

Four major dimensions of the self-image were considered in this analysis: (1) self-consciousness, i.e., the salience of the self to the individual; (2) stability of the self-image; (3) self-esteem, i.e., the individual's global positive or negative attitude toward himself; and (4) the perceived self, i.e., the individual's perception of significant others' opinions of him. Self-consciousness, stability, and self-esteem were measured by Guttman scales possessing acceptable Reproducibility and Scalability Coefficients. The "perceived self" indicators were not summated since each individual has a number of perceived selves.

The results clearly suggest that the emergence of self-image problems in adolescence is no myth, and that these problems occur early in adolescence. In general, self-image disturbance appears to be much greater in the 12-14 age group than in the 8-11 age group. In contrast to younger children, the early adolescents (12-14 year olds) show a higher level of self-consciousness, greater instability of the self-image, slightly lower global self-esteem, and a more negative "perceived self." (All these differences are statistically significant at the .01 level.) Early adolescents also show a higher level of depressive affect than do the younger children. The only area of improvement involves the opposite sex: children see themselves as better liked by the opposite sex as they grow older.

In general, the various dimensions of the self-image appear to improve somewhat in later adolescence (15-18 year olds) although the respondents still manifest greater self-consciousness and instability than do the 8-11 year old children. Only global self-esteem is higher in later adolescence. On the other hand, the younger children seem to be consistently more likely to say that they are performing well in terms of their self-values.

Is it possible to pin down the time of onset of disturbance more precisely? According to our data, the sharpest rise in disturbance appears during the twelfth year (i.e., between the twelfth and thirteenth birthdays). With regard to some dimensions, this sharp increase continues through the twelfth and thirteenth years. After age 13, there is a general levelling off. In general, adolescence--the twelfth and thirteenth years--appears to be characterized by a more dramatic rise in self-image disturbance than at any other point in time.

While the time of onset in self-image disturbance strongly suggests that puberty is of major importance, there is one environmental factor which characteristically intrudes at this life-stage, namely, the transition from elementary to junior high school. The question thus arises: are the 12 and 13-year olds more disturbed because they are older or because they have entered junior high school? One indication is offered by comparing 12-year olds in sixth and seventh grades. The data show that 12-year olds in junior high school have lower global self-esteem, higher self-consciousness, and greater instability of the self-image than their age-peers in elementary school. These results are maintained when race, social class, and marks in school are held constant.

Further evidence that it is the transition from elementary to junior high school, rather than simply the biological aging process, appears when we compare the self-images of 11 and 12-year olds in the sixth grade and 12 and 13-year olds in the seventh grade. Within each grade there are virtually no self-image differences among the children differing in age. Otherwise expressed, within the same school class, age makes little difference, but within the same age grouping, school class makes a large difference.

It should be noted, incidentally, that the transition from junior to senior high school does not show a parallel effect on the self-image. The onset of puberty in itself may thus not generate self-image disturbance but it may give rise to a heightened vulnerability to stressful environmental circumstances which threaten the self-concept.

(2) Significant others--A different line of analysis has focused on "significant others." The theory of reflected appraisals--the idea that the individual comes to see himself as he perceives others see him--has been advanced in different ways by such diverse thinkers as James, Mead, Cooley, and Sullivan. While the empirical evidence clearly supports this theoretical position, there are substantial discrepancies. Some people who believe others think well of them have negative self-attitudes, and vice versa.

The springboard for this analysis was our hypothesis that it was not only the attitude of the other, but also the attitude toward the other, that might affect the self-image. The assumption tested was a simple but neglected one: that not all significant others are equally significant.

Two aspects of significance were considered: valuation and credibility. The first deals with whether the individual cares what the other thinks of him, the second with whether he trusts or respects the other person's judgment.

Seven putative significant others were considered: mother, father, teachers, kids in class, boys, girls, and siblings. While the assumption of reflected appraisals was supported in each case, valuation improved prediction and understanding. With regard to six of these seven putative others, the association between the respondent's perception of the other's attitude and the respondent's own self-esteem was stronger if he valued the other's opinion than if he did not. For example, the child's view of what his father thought of him and what he thought of himself was strong if he cared what his father thought but weak if he was indifferent to his father's opinion.

This finding led to the obvious question: why are some others especially significant, others less so? In part this is a reflection of the individual's immersal in the social system, but in part it appears to involve the use of a defense mechanism--what we have called selective valuation. While valuation of the favorable opinions of others is bounded by social reality (almost all children care most about their mothers' opinions of them), there is also an element of volition involved. What our data show is that the child is strikingly more likely to decide that he cares about the opinions of those who, in his view, think well of him, and to be relatively unconcerned with the opinions of his detractors.

The other aspect of significance is credibility. It is extremely interesting to note how much the locus of self-knowledge in children is external to themselves. Children are more likely to believe that their mothers know better than they themselves how smart, good, or good-looking they are. Indeed, nearly half think that some other individual knows better what they "are like deep down inside" than they themselves. As one would expect, the younger the child, the greater his faith in an external authority about what he is like.

The importance of source credibility for the self-concept is shown in our data. In each case, the relationship between the perceived self and self-esteem turns out to be stronger if the other has high credibility than if he has low credibility. But who has high credibility in the eyes of the child? As expected, credibility is in part determined by the motivation to protect and enhance the self-concept. Our data clearly show the operation of "selective credulity": the children tend to give greater credence to those believed to appreciate their merits than to those more alert to their shortcomings.

These data indicate that the theoretical principle of reflected appraisals is in need of specification and refinement. Furthermore, investigators must recognize that significance is in the eye of the subject and cannot be prejudged by the investigator.

While motivational factors contribute to the determination of significance, the individual's location in the social structure, especially his "role-set," plays a much larger role. Our data show that both valuation and credibility are affected by the individual's groups, statuses, or social categories--his age, race, sex, and socioeconomic status. Both social structure and psychological process conjoin to determine who will be more or less significant to the individual and thereby exercise a corresponding influence on his self-image.

Scientific Significance: Problems of the self-image are often involved in mental health and illness. Yet little is currently known about how position in the social structure, and the characteristic experiences associated with this position, influence the formation of the self-image. This study is designed to contribute knowledge to this area, and to learn how the self-image changes with age.

Proposed Course of Project: Data analysis will continue through the coming year. One area we plan to investigate is the child's ego-extensions. The self incorporates all those elements which engage the individual's self-feeling, including various aspects of his social identity. We plan to investigate the significance of the individual's location in the social structure for the determination of his specific ego-extensions. Another area of investigation will involve the study of identification. Our analysis will seek to provide some of the specific information so frequently lacking in this area: who identifies or disidentifies with whom in respect to what qualities, what factors account for these identification patterns, how do these identification patterns change as children grow older, and what is the connection between identification and current self-concept. Finally, we plan to give further consideration to the significance of dissonant and consonant social contexts for the self-image.

Honors and Awards: None

Publications:

Rosenberg, M.: Which significant others? American Behavioral Scientist, in press.

Simmons, R. G., Rosenberg, F., and Rosenberg, M.: Disturbance in the self-image at adolescence. American Sociological Review, in press.

Rosenberg, M.: The logic of suppressor variables. Public Opinion Quarterly, in press.

Serial No. M-S-SP-4
1. Socio-environmental Studies
2. Social Process
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Individual Differences in Survival and Reproduction Among Old Colony Mennonites in Mexico

Previous Serial Number: Same

Principal Investigators: Gordon Allen and Calvin Redekop (non-PHS)

Other Investigators: None

Cooperating Units: Goshen College, Goshen, Indiana

Person Years

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: To demonstrate the presence or apparent absence of selective survival and reproduction with respect to individual behavioral characteristics. Also to study natural patterns of marriage and reproduction in a relatively simple agricultural society of traditional European type.

Methods Employed: The population under study consists of about 13,000 German-speaking Mennonites in a politically integrated cluster of villages in northern Mexico. These people were selected because of their simple and uniform culture insulated from mass communication media and because of their apparently unrestricted fertility. Also important was the fact that, unlike some similar religious isolates, this society supports a considerable degree of economic competition.

Information was gathered by means of a combined census and questionnaire that reached a third of the population. This has been supplemented with photographs of all vital records kept by the church, a public list of farmers and their holdings, a list of all village and church leaders in the 40-year history of the population, and records of Mennonite births in local hospitals. Interviews were conducted by the investigators and by a Canadian Mennonite with the aid of cooperative local men. This material defines the background of attitudes toward community problems, overpopulation, and birth control.

The main data sets have been completely edited for accuracy and consistency. Analysis of church records by tabulation methods has proceeded as far as is practicable before linking them to the census, which will be the next big step. This will permit verification of information on about a third of the living population and analysis of relations of past vital events to social and economic data obtained with the census.

Major Findings: Men who have held public office in the Old Colony appear to have more children than other men. If election to office is any measure of social competence, this finding contrasts with the usual pattern in recent industrial societies and indicates how natural selection may have maintained or improved mental qualities in advanced agricultural societies. However, it is possible that one of the requirements for public office in the Old Colony is marriage and the fathering of children. We have some evidence against this, but the question is being pursued.

Women who bore a child before marriage appear to be at a disadvantage in getting married, or they are more often married into another colony. But those who do marry appear to have more children than the average, even when their illegitimate child is excluded.

Scientific Significance: Natural selection is apparently the sole agent responsible for the progressive evolution and maintenance of human mental capacities. The study of selection in a pre-industrial European society may indicate some of the influences that have produced present genetic tendencies and draw a base line for future studies of selection under modern conditions.

Proposed Course of Project: Analysis of the data is expected to occupy between two and four years.

Honors and Awards: None

Publications: None

Serial No. M-S-SP-5

1. Socio-environmental Studies
2. Section on Social Process
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Social Origins of Stress

Previous Serial Number: Same

Principal Investigator: Leonard I. Pearlin

Cooperating Units: None

Person Years

Total:	2 3/4
Professional:	1
Other:	1 3/4

Project Description:

Objectives: This study examines the interrelations between three aspects of social and psychological life: the recurring problems and strains that people experience in their everyday lives, their efforts to deal with such experiences, and the subjective states of stress that may result. Much of the inquiry is organized around the strains that occur in the major role areas of work, economic maintenance, parenthood and marriage. In looking at the potentially stressful experiences that people have as they act within these institutional roles, we shall be comparing those of different socio-economic class, of different race, sex and age ranks. In this way we shall be pursuing a foremost goal of the investigation: to observe the relationships of elements of social structure to enduring strains, to coping repertoires, and to states of stress.

Methods Employed: The data have been gathered from a probability sample of 2300 households in the Chicago urbanized area. An adult member from each household was interviewed with a standardized schedule developed over a two-year period.

Major Findings: None. The past year has been spent in preparing the final interview schedule, drawing a sample, recruiting and training interviewers, completing the survey, coding and verifying the data, and creating and editing a computer tape.

Scientific Significance: The significance of this study derives from several of its features. First, no other single study of stress has brought together as large an array of variables. This will enable us to deal in a more encompassing fashion with the complex relationships involved in the processes by which the inner lives of people are affected by the social environment in which they are located. Second, it emphasizes recurrent, structured experiences as possible antecedents of stress rather than eruptive and transitional events. Third, it treats coping less as individual devices than as practices learned from and shared with others similarly located in the social structure. Fourth, the study has a broad relevance to mental health issues. The relationships of class, sex and family to psychiatric symptomatologies are firmly established, yet there is a striking absence of studies bearing on the dynamics underlying such relationships. This study should help to fill the void.

Proposed Course of Project: It is expected that the data tape will be prepared for analysis early in 1973 and that analyses will immediately follow. The general strategy of analysis will compare groups located in different parts of the social structure with regard to the strains they experience, their coping reactions, and the stress with which they are left.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-1
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neural substrate of mirror display in squirrel monkey (*Saimiri sciureus*).

Previous Serial No.: M-NP-LI-23

Principal Investigators: Paul D. MacLean

Other Investigators: Robert E. Gelhard

Cooperating Units: None

Man Years

Total: 1.4
Professional: 0.4
Other: 1.0

Project Description:

Objectives: This long-term study utilizes the innate display behavior of the squirrel monkey (1) for obtaining information about the cerebral representation of species-typical behavior and associated imitative factors, and (2) identifying structures involved in the integration of autonomic and somatic functions in visually guided sociosexual behavior. The present experiments continue investigation of effects of bilateral lesions of the globus pallidus and its main efferent pathways.

Methods Employed: The mirror display test for male gothic type squirrel monkeys has been described in previous project reports (M-NP-LI-17, 1963 and 1964). After animals reach criterion in 30 or more trials, they are subjected to bilateral coagulation of different parts of the globus pallidus and retested for several weeks or months. Drawings are made of serial histological sections for locating the lesions and calculating the amount of destruction by planimetry.

Major Findings:

Pallidal lesions. A detailed analysis has now been made on fourteen animals with electrocoagulative lesions in different parts of the globus pallidus. A statistically significant, enduring deficit in display occurred in 10 (70%) of the animals. Regardless of locus, small bilateral lesions resulted in no lasting deficit, even when as many as six months were allowed to elapse for further degenerative changes to occur. Additional lesions, however, may result in an abrupt and enduring deterioration of performance. In general, the factor of physiological safety (Meltzer) applies insofar as performance is unaffected unless tissue destruction or cell loss approaches a critical amount. A possible exception pertains to medially placed lesions, which in addition to the pallidal tissue, destroy a part of the efferent pathways.

Monkeys with anterior or middle lesions usually require hand-feeding during the acute post-operative period (2 to 11 days). All animals except three, however, maintained or bettered their weight during the long post-operative period.

In the course of this work it has been shown that bilateral interruption of the stria terminalis, Arnold's bundle, the amygdalofugal pathway, and the destruction of the greater part of the nucleus of the ansa peduncularis do not interfere with the full expression of the display. The same applies to Wernicke's area and the posterior part of the internal capsule.

Pallidofugal pathways. It was known from a previous experiment on a previous animal (Z-3) that massive destruction of pallidofugal pathways eliminated display behavior. An attempt is now being made to discover which parts of the pallidal projections are most crucial for the display, as well as to distinguish the functions of these pathways from those of the medial forebrain bundle. Thus far lesions have been placed in two animals which are still being tested.

Significance to Biomedical Research and Program of the Institute. Despite 150 years of experimentation, little is known about the functions of the striatal complex. The present experiments are significant because they show that, contrary to traditional views, the striatal complex is not simply a motor mechanism, but plays a basic role in species-typical forms of behavior. Because of the imitative factors involved in the display, the study also gives important leads about forebrain mechanisms involved in imitation, a subject about which virtually nothing is known.

Proposed Course of the Project: To be continued.

Honors and Awards: Items pertaining to Dr. MacLean are as follows:

1) Invited Speaker: "Evolutionary Trends of the Triune Brain: From Imitation to Creativity," International Colloquium on "The Unity and Diversity of Man," Centre International d'Etudes Bio-Anthropologiques et d'Anthropologie Fondamentale, Royaumont Abbey, France, September 6-9, 1972.

2) Invited Member of Commission of the 52nd Annual Meeting of the Association for Research in Nervous and Mental Disease, Subject: Aggression. Hotel Roosevelt, New York, N.Y., December 1-2, 1972.

3) Invited Panelist: Panel Discussion on Psychosurgery, Jack Masur Auditorium, Clinical Center, National Institutes of Health, Bethesda, Maryland, January 18, 1973.

4) Invited Lecturer: "Neurochemical Distinctions of Our Triune Brain." Division of Neuropharmacological Drug Products, Food and Drug Administration, DHEW, Parklawn Building, Rockville, Maryland, February 8, 1973.

5) Invited Lecturer: "Brain Mechanisms of Ancestral Behavior." Center for Neurobiological Sciences, University of Florida, Gainesville, Florida, March 17, 1973.

6) Invited Chairman, Physiology Session on Behavior, 57th Annual Federation of American Societies for Experimental Biology in Atlantic City, N.J. on April 18, 1973.

7) Invited Lecturer: "Brain Mechanisms of Ritualistic Behavior," Colloquium of the Department of Psychology, University of Virginia, Charlottesville, Virginia, May 11, 1973.

8) Invited Speaker: "Influence of Limbic Cortex on Hypothalamus: New Anatomic and Microelectrode Findings." International Symposium on Recent Studies of Hypothalamic Function, Medical School of The University of Calgary, Alberta, Canada, May 29-31, 1973.

9) Invited Speaker: "Sensory and Perceptive Factors in Emotional Functions of the Triune Brain." International, Interdisciplinary Symposium on "Parameters of Emotion," Karolinska Institute, Stockholm, Sweden, June 4-6, 1973.

Publications:

MacLean, P.D.: Cerebral evolution and emotional processes: New findings on the striatal complex. Ann. NY Acad. Sci. 193: 137-149, 1972.

MacLean, P.D.: New findings on brain function and socio-sexual behavior. In Zubin, J. and Money, J. (eds.): Contemporary Sexual Behavior: Critical Issues in the 1970s. Baltimore, Md., The Johns Hopkins University Press, 1973, pp. 53-74.

MacLean, P.D.: The limbic galaxy: Honoring a brain astronomer at Graz. In Heppner, F. (ed.): Limbisches System and Epilepsie. Aktuelle Probleme in der Psychiatrie, Neurologie, Neurochirurgie: 9. Bern, Verlag Hans Huber, 1973, pp. 52-61.

Poletti, C.E., Kinnard, M.A. and MacLean, P.D.: Hippocampal influence on unit activity of hypothalamus, preoptic region, and basal forebrain in awake, sitting squirrel monkeys. J. Neurophysiol. 36: 308-324, 1973.

MacLean, P.D.: An evolutionary approach to the investigation of psychoneuroendocrine functions. Proceedings of the Second Congress of the International Society of Psychoneuroendocrinology, (in press).

MacLean, P.D.: A triune concept of the brain and behavior, Lecture I. Man's reptilian and limbic inheritance; Lecture II. Man's limbic brain and the psychoses; Lecture III. New trends in man's evolution. In Boag, T. and Campbell, D. (eds.): The Hincks Memorial Lectures. Toronto, University of Toronto Press, (in press).

MacLean, P.D.: Evolutionary trends of the triune brain: From imitation to creativity. In Royaumont Symposium on "The Unity and Diversity of Man" (in press).

MacLean, P.D.: The brain's generation gap: Some human implications. Zygon J. of Relig. & Sci. (in press).

Serial No. M-LBEB-CN-8
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neural substrate of olfaction in the rat.

Previous Serial No.: M-LBEB-CN-8

Principal Investigators: Burton M. Slotnick

Other Investigators: Howard M. Katz and Robert E. Gelhard

Cooperating Units: None

Man Years

Total:	1.5
Professional:	0.5
Other:	1.0

Project Description:

Objectives: These experiments utilize an operant conditioning procedure for analyzing the role of basal rhinencephalic structures in olfactory discrimination.

Methods Employed: Prior to surgery rats were trained in a wind-tunnel olfactometer (see Report M-NP-LI-50, 1971) to report detection of a 1-second odor sample of dilute amyl acetate. Following extensive overtraining the olfactory bulbs (N=3), lateral olfactory tracts (N=6), anterior limbs of the anterior commissure (N=3) or anterior amygdala (N=3) were destroyed in different groups of animals. In two rats both the anterior commissure and lateral olfactory tracts were sectioned. Three animals with neocortical lesions served as controls. Following postoperative retention tests, animals were trained on a series of 2-odor discriminations of increasing difficulty.

Major Findings: Animals with bilateral removal of the olfactory bulbs showed no retention and were unable to reacquire the odor discrimination. Identical results were obtained for one rat in which the olfactory peduncles were transected. Rats with bilateral section of the lateral olfactory tracts (LOT) had no retention, but were able to reacquire the odor discrimination after extensive retraining. Animals with the most anterior LOT lesions required the greatest number of retraining trials. All LOT subjects showed moderate to severe deficits in the 2-odor discriminations and the magnitude of the impairment was related to both the frontal level of the lesion and the difficulty of the discrimination. Little or no impairment was observed in animals with bilateral section of the anterior commissure, lesions of the anterior amygdala, or neocortex. Of the two animals with combined LOT and anterior commissure lesions one showed only moderate deficits and the other a total inability to detect odors. In the former, the LOT transection was made posteriorly, at the level of the olfactory tubercle; in the latter, destruction of the LOT was complete at the level of the caudal portion of the olfactory bulb.

Significance to Biomedical Research and Program of the Institute. Although olfactory pathways represent the primary exteroceptive input to the limbic system, no systematic study has been made of their role in olfactory discrimination. The present results demonstrate that anterior section of the LOT produces severe deficits in olfaction but not anosmia and strongly suggests the existence of a medial afferent pathway, perhaps via the anterior commissure. In contrast, the lack of effect on olfactory discrimination by destruction of projection sites in the amygdala indicates that this limbic structure may have other olfactory functions than simple detection or discrimination of odors.

Proposed Course of the Project: To be continued.

Honors and Awards: Items pertaining to Dr. Slotnick are as follows:

1) Invited Lecturer: "The Limbic System." Contribution to the Fourth Summer Workshop in Neuropsychopharmacology for Senior Graduate Students. Department of Pharmacology, Vanderbilt University School of Medicine, June 14, 1972.

2) Invited Lecturer: "The Forebrain Limbic System: Anatomy and Function." Department of Anatomy, Howard University Medical School, October 17, 1972.

3) Invited Lecturer: "Neural Substrate of Olfaction." Department of Anatomy, Georgetown University, December 19, 1972.

Publications: None

Serial No. M-LBEB-CN-15
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Unit analysis of vagal inputs to the diencephalon. I. Intralaminar nuclei.

Previous Serial No.: None

Principal Investigators: Robert A. Hallowitz and Paul D. MacLean

Other Investigators: Patrick J. Carr and Henry Waite

Cooperating Units: None

Man Years

Total:	1.4
Professional:	1.1
Other:	1.3

Project Description:

Objectives: Despite the recognized importance of visceral influences on cerebral function little is known about interoceptive inputs to the three main evolutionary formations of the brain. The present study utilizes an improved technique for vagal stimulation in chronically prepared monkeys to test the effects of vagal volleys on units of intralaminar nuclei.

Methods Employed: Extracellular unit activity is recorded by glass micropipettes (5-10 MΩ) in squirrel monkeys with a chronically fixed stereotaxic platform. Bipolar, concentric, stainless steel electrodes are chronically implanted in the jugular foramen next to the vagus and are led to a female plug on the platform.

Major Findings: To date 155 units have been tested in n. medialis dorsalis, parafascicularis, centrum medianum, and centralis lateralis. Of 120 units in medialis dorsalis 31 (26%) were affected by vagal volleys, with 17 (14%) showing initial excitation and 14 (12%) initial inhibition. Three

(15%) of 20 units in n. centrum medianum and 1 (14%) of 7 in n. centralis lateralis showed initial excitation. Response latencies ranged from 20-90 msec.

Significance to Biomedical Research and Program of the Institute. Visceral symptoms may be a prominent feature in psychoneurotic, psychosomatic, and psychotic illnesses. Severe visceral symptoms are a dreaded part of the withdrawal manifestations in narcotic addiction, adding to the difficulties of enlisting the cooperation of patients in submitting to treatment. The improved techniques of the present study promise to provide much needed information about the cerebral representation of visceral functions.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-16
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effects of destruction by 6-hydroxydopamine of nigrostriatal system in squirrel monkey (*Saimiri sciureus*).

Previous Serial No.: None

Principal Investigators: Paul D. MacLean and Robert A. Hallowitz

Other Investigagors: Robert E. Gelhard and Henry Waite

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description:

Objectives: The present study attempts to learn whether or not ascending dopaminergic systems from the midbrain can be selectively destroyed by 6-hydroxydopamine (6-OHDA).

Methods Employed: Two squirrel monkeys were used in this pilot study. In one case, 0.02 µl of a solution containing 20 µg of 6-hydroxydopamine was stereotaxically injected near the medial aspect of the cerebral peduncle between F7 and F5. In the other case the agent was deposited in solid form according to a method described by MacLean in 1957.

Major Findings: In the animal injected with solution, flexor rigidity of the somatic musculature was evident before it recovered from anesthesia. Upon awakening, the animal was cataleptic and this condition gradually changed to a picture resembling parkinsonism. It was almost totally incapacitated and required feeding. Its condition was only partly helped by treatment with L-dopa and MK-486. The manifestations in the other animal were essentially similar, except that treatment with L-dopa resulted in a temporary improvement in mobility and the capacity to feed itself. Motion pictures were taken to document the findings in each case.

Autopsy revealed necrosis at the site of application of 6-OHDA, with damage being more extensive in the case in which the chemical was deposited in solid form. In each case there was tissue damage along the needle track, resulting from reflux of the chemical. There was obliteration in each case of the nerve cells in the ventral tegmental area and medial part of the substantia nigra. In the animal injected with solution, there was virtual disappearance of the cells of pars compacta of the substantia nigra.

Significance to Biomedical Research and Program of the Institute. The destruction of the medial part of substantia nigra and the ventral tegmental area, which presumably interrupts ascending dopaminergic pathways to the olfactory striatum and corpus striatum, results in a condition resembling parkinsonism in the squirrel monkey. The necrotizing action of 6-OHDA makes it unsuitable for selectively destroying dopaminergic cells and fibers.

Proposed Course of the Project: Concluded.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-17
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Comparative study of aminergic systems of the brain.

Previous Serial No.: None

Principal Investigators: David M. Jacobowitz and Paul D. MacLean

Other Investigators: None

Cooperating Units: Laboratory of Clinical Science
National Institute of Mental Health

Man Years

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: Most fluorescence studies on the cerebral location of cell bodies and terminals of aminergic systems have been done on the rat. The present study is undertaken to learn if corresponding systems exist in primates.

Methods Employed: The fluorescent technique of Falck and Hillarp is used for comparing the brains of mouse, rat, pygmy marmoset, and squirrel monkey. In selected cases tranylcypromine is administered for enhancing the fluorescence of serotonin containing elements.

Major Findings: Essentially the same groups of norepinephrine, dopamine, and serotonin containing cells have been found in the monkey, as originally described in the rat by Dahlström and Fuxe. With allowance made for minor differences, the same statement applies to terminals. In the pygmy marmoset there do not appear to be as many green fluorescent cells in the nucleus solitarius as in the rat. There is a distinctive crescent-shaped distribution of terminals in the ventrolateral part of the dorsal vagal nucleus. Except for minor differences, the picture of fluorescence in locus ceruleus of the squirrel monkey and marmoset is similar to that in the mouse and rat (Group A6). Some cells in this region can be followed into the commissure of the isthmus, and are also seen within fascicles of the fourth nerve.

The yellow fluorescent cells (serotonin) of the fountain nucleus are equally prominent in the marmoset as in the rat (Group B7), as are the surrounding green terminals. The yellow fluorescence of the dorsal part of the interpeduncular nucleus is more conspicuous than in the rat, resembling a rising full moon. Serotonin containing cells above this nucleus, as well as those within and bordering the medial lemeniscus at this level correspond to Groups B8 and B9 in the rat. Yellow fluorescence is seen just lateral to the mammillary bodies.

In both the marmoset and the squirrel monkey cells smaller than those of zona compacta of the substantia nigra are found among the fascicles of the third nerve and in the ventral tegmental area. These presumably correspond to the dopamine containing cells of Group A10 of the rat. A degeneration study on the course of the ascending dopamine containing nigrostriatal system in the squirrel monkey was described in last year's report (M-LBEB-CN-7). Additionally, it should be mentioned there are some presumably norepinephrine containing processes that follow the ansa lenticularis into the sublenticular region. There is a conspicuous group of green fluorescent cells dorsal to the third ventricle in the region of passage of the mammillo-thalamic tracts. The picture of green fluorescent terminals in the central gray, habenular region, caudal pole of thalamus, hypothalamus, preoptic area, and septal region is essentially similar to that seen in the rat. In the marmoset there is a conspicuous collar of terminals around the fornix at the level of the anterior hypothalamus. The olfactory striatum and corpus striatum show the same type of diffuse, felt-like fluorescence (dopamine) seen in the mouse and rat.

Green fluorescent terminals are seen in strata oriens et radiatum of areas CA4 and CA3 of the hippocampus.

The marmoset is unlike the rat in having norepinephrine containing fibers within the walls of the intracerebral vessels.

Significance to Biomedical Research and Program of the Institute. There is now substantial evidence that the ascending dopamine striatal systems from the midbrain play a fundamental role in "setting the tone" for behavioral activities of the organism. The serotonin systems have been implicated in sleep, and some evidence suggests that the norepinephrine containing systems play a role in modulating acetylcholine mechanisms. The comparative findings of the present study indicate that the organizational pattern of these phylogenetically ancient aminergic systems has been preserved with remarkable consistency in the evolution of primates.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-18
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Comparative survey of behavioral changes induced by apomorphine.

Previous Serial No.: None

Principal Investigators: Paul D. MacLean

Other Investigators: Robert E. Gelhard

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.1
Other:	0.2

Project Description:

Objectives: It is now believed that one of the major sites of action of apomorphine is on dopamine receptors. The only known dopamine receptors in the forebrain are those associated with the olfactory striatum and the corpus striatum which receive projections from dopamine containing cells in the ventral tegmental area and substantia nigra. As background for comparative investigations of this Section on the functions of the striatal complex, it is desirable to have information about the general behavioral effects of dopamine on a variety of species.

Methods Employed: Apomorphine is administered parentally in doses of 1 mg/kg to animals under caged and uncaged conditions. Thus far observations have been made on the parrot, turkey, opossum, and squirrel monkey.

Major Findings: In all the species treated, apomorphine appears to result in increased aimless activity. Within a few minutes the parrot begins to have squawking vocalization accompanied by side-to-side and up-and-down movements of the head. The squawks first occur in pairs, then threes, then fours, then fives, until at the height of the drug's action (about 20 minutes) they are continuous. The symptoms gradually subside in the next half hour. The turkey develops aimless running in and out of the flock and will stop only momentarily to retaliate to the aggressive behavior aroused in members of the flock. This behavior may continue as long as three to four hours. During this time the snood shows an unusual degree of relaxation. The opossum, a nocturnal animal, arouses from its daytime repose and hurries helter skelter about its habitat, mindless of a drinking trough or anything else in its way. This effect lasts less than a half hour. If in their cages, squirrel monkeys develop a kind of "obstinate progression" in which they keep pushing their heads against the top of the cage. The behavior is similar to the "obstinate progression" described by Mettler in macaques with large striatal lesions. In addition, the squirrel monkey may bite against the sides of the cage and show dyskinetic movements of the limbs. Outside the cage it moves about in a restless, disoriented manner, and contrary to its usual behavior, may approach and attempt to bite the observer.

Significance to Biomedical Research and Program of the Institute. Snyder has recently advanced arguments for inferring that the ascending dopaminergic systems to the olfactory striatum and corpus striatum are implicated in amphetamine psychosis. The comparative observations described here suggest that these systems exert an energizing influence on an animal's activities. This inference is supported by the finding that destruction of the ascending dopaminergic pathways (see M-LBEB-CN-11) results in almost total incapacitation.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-19
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Olfactory stimulus control.

Previous Serial No.: None

Principal Investigators: Burton M. Slotnick and Howard M. Katz

Other Investigators: Robert E. Gelhard and Patrick J. Carr

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.3
Other:	0.6

Project Description:

Objectives: Little information exists concerning the use of olfactory cues in learned behaviors. The present experiments tested the ability of rats to form a learning set based on olfactory cues and examined the differential utilization of olfactory and visual cues.

Methods Employed: Rats were trained using a "go, no-go" procedure to discriminate odorous or visual stimuli. The apparatus and training procedures used have been described in Project Report M-NP-LI-50, 1971.

In Experiment 1, eight rats first trained to discriminate visual cues were given a series of 15 2-odor discrimination problems. Inter-problem improvement in performance provided a measure of learning set ability. The odor stimuli were floral perfumes and members of each pair had been matched by a perfumist for similarity of odor quality and intensity.

In Experiment II, 2 groups of 4 rats were first trained to respond to a S-plus odor or visual stimulus following which the

S-minus stimulus was introduced. After discrimination criterion had been achieved, all animals were given further training during which the odor or visual stimuli was added to the stimuli being discriminated (compound stimulus training). The original training stimuli were then removed and training continued until criterion performance for the remaining stimuli had been achieved.

Major Findings: In Experiment I all rats showed a dramatic improvement in performance over the 15 odor discrimination problems. By the end of training most animals made only 2-6 errors before reaching performance criterion.

In Experiment II animals first trained on the S-plus visual stimuli required several hundred trials to reach discrimination criterion after introduction of the S-minus visual stimulus. Animals trained on the S-plus odor reached discrimination criterion after only 3-10 errors following the introduction of the S-minus odor. Performance following compound stimulus training demonstrated that animals initially trained on odors showed little or no acquisition of the visual discrimination during the compound trials. In contrast, animals initially trained on visual stimuli acquired the odor discrimination during the compound training.

Significance to Biomedical Research and Program of the Institute. These experiments demonstrate that olfactory cues are preferentially attended to by the rat and afford the acquisition of learning sets at levels comparable to those achieved by primates in response to visual stimuli. The results clearly support an attentional theory of discrimination learning and, in studies of comparative learning abilities, emphasize the need to consider the stimulus in relation to its functional significance for the subject.

Proposed Course of the Project: Terminated.

Honors and Awards: Item pertaining to Dr. Slotnick is as follows:

1) Invited Lecturer: "The Sweet Smell of Success: Recent Studies on Olfactory Discrimination Learning." Department of Psychology, Illinois Institute of Technology, February 5, 1973.

Publications: None

Serial No. M-LBEB-CN-20
1. Laboratory of Brain
   Evolution and Behavior
2. Section on Comparative
   Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Aggression and defense in mice: Behavior following septal forebrain lesions in a social group of mice.

Previous Serial No.: None

Principal Investigators: Burton M. Slotnick and Halsey M. Marsden

Other Investigators: None

Cooperating Units: Section on Behavioral Systems, Laboratory of Brain Evolution and Behavior

Man Years

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description:

Objectives: Previous studies have shown that after septal lesions mice show hyper-reactivity, as well as deficits in aggressive, maternal, and learned behaviors. The question arises as to what extent the deficits are attributable to the restrictions of usual laboratory test procedures. The present experiment was designed to examine the effects of septal lesions on aggressive and social forms of behavior in a group of mice living in a simulated natural environment.

Methods Employed: Two groups of 12 male mice were housed separately in 4-cell "Calhoun" mouse universes and observed for several months prior to and following surgery. All members of one group received septal lesions while the other group served as sham-lesioned controls. After a 7-day recovery the groups were reconstituted. Additional variables included the introduction of females for a brief period and combining the two groups in a single enclosure.

Major Findings: Prior to surgery both groups showed a high degree of social organization including nest building, territorial defense, and dominance hierarchies. Postoperatively no changes in behavior were observed in the control group. Mice with septal lesions showed fragmented aggressive behavior on the first postoperative day but social organization rapidly returned to the stable control level within a few days. Following introduction of females experimental mice showed few attempted and, only occasionally successful, copulatory acts and inappropriate aggression toward females but behavior improved to control levels after 1-2 days. When the two groups were housed together, septal lesioned mice initially engaged in little or no aggressive behavior and no territorial defense. However, after 3-4 days experimental animals were observed fighting and eventually territorial zones were established and defended equally well by both control and lesioned mice. In contrast to these transient changes in social behavior, mice with septal lesions did not build nests and this deficit was permanent.

Significance to Biomedical Research and Program of the Institute. Although previous reports have emphasized changes in aggressive behavior in mice following septal lesions, the present results demonstrate that such changes are transitory and do not represent deficits in the ability to express normal aggressive behavior, defend territorial zones, and establish stable social organization. These results emphasize the importance of the physical environment, with its critical variables of complexity and time, in studies of brain mechanisms underlying social behavior.

Proposed Course of the Project: Terminated.

Honors and Awards: Items pertaining to Dr. Slotnick are as follows:

1) Invited Speaker: "Olfaction Two Years Later: Implications for Comparative Psychology." The Neuroscience Seminar Series, Laboratory of Neurophysiology, NIMH, January 19, 1973.

2) Invited Lecturer: "Aggression and Current Concepts of Limbic Function." Department of Biophysics and The Graduate School, Ohio State University, February 20,1973.

3) Invited Lecturer: "Olfaction and Stimulus Control of Discriminative Behavior." Monell Chemical Senses Center, University of Pennsylvania, March 13, 1973.

Publications:

Slotnick, B.M., McMullen, M.F. and Fleischer, S.: Changes in emotionality following destruction of the septal area in albino mice. Brain, Behavior and Evolution, (in press).

Slotnick, B.M. and McMullen, M.F.: Response inhibition deficits in mice with septal, amygdala or cingulate cortical lesions. Physiology and Behavior, (in press).

Serial No. M-LBEB-CN-22
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mediation of amygdala in conditioned gustatory aversion.

Previous Serial No.: None

Principal Investigators: David L. Brown and Burton M. Slotnick

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: Although conditioned gustatory aversion is now well established as a biologically important and unique form of learning underlying the well known "bait shyness" effect, little is known concerning its neurological basis. Since experimentation has implicated the amygdala in the control of autonomic function, gustation, and avoidance reactions, the present experiment examines the effects of amygdala lesions on the acquisition of a gustatory aversion.

Methods Employed: Four rats with bilateral amygdala lesions and 4 rats with small neocortical lesions were placed on a 23-3/4 hour water deprivation schedule. On days 6, 11, 14, and 17 a 0.1% sodium saccharin solution was substituted for the usual drinking water. Twenty minutes following exposure to the saccharin solution each animal was injected with 6 mg/kg of apomorphine. Additional saccharin tests were given on days 19, 21, and 23. Two additional animals with amygdala lesions served as saline-injected controls.

Major Findings: Table 1 presents the saccharin aversion scores (percentage change in intake on test days relative to tap water intake) for the 4 conditioning and 2 extinction tests for each group. Negative scores indicate a reduction in intake and positive scores an enhancement on saccharin test days.

	Treatment-Test Days				Extinction Days	
	1	2	3	4	1	2
Neocortical	-36	-33	-33	-48	-26	-09
Amygdala	04	07	08	15	30	30
Amygdala Control	10	00	26	33	29	29

As a result of saccharin-apomorphine pairing, animals with neocortical lesions showed a strong aversion to saccharin, with a decrease of their total fluid intake of 33-48% on test days. Recovery close to base level was observed during the two extinction tests. In contrast, the amygdala group developed no aversion for saccharin. However, a weak conditioned effect in this group is suggested by reduced saccharin intake during conditioning tests relative to the intake by amygdala control rats and by a marked increase during the extinction tests.

Significance to Biomedical Research and Program of the Institute. Previous reports by Garcia and by Berger have suggested that bait shyness represents a unique and primitive form of learning mediated primarily by medullary gustatory and visceral afferent systems. Although the anatomical examination has not been completed, the present results suggest that these illness-induced aversions involve the limbic system and provide additional evidence for the role of the amygdala in the conditioning of visceral afferent systems.

Proposed Course of the Project: Terminated.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-23
1. Laboratory of Brain
   Evolution and Behavior
2. Section on Comparative
   Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Olfactory cues in illness-produced conditioned aversion.

Previous Serial No.: None

Principal Investigators: David L. Brown and Burton M. Slotnick

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: Bait shyness appears to be a unique form of learning in which a taste serves as a conditioned stimulus for illness following ingestion of a novel food. Previous studies have not adequately tested the role of olfaction or controlled for olfactory cues in mediating bait shyness. The present study was designed to learn whether or not olfactory cues can serve as conditional stimuli for drug induced illness.

Methods Employed: Four rats, maintained on a water deprivation schedule, were trained in an olfactometer to perform a simple operant response for a water reward. During 15-minute training sessions, each animal was given, on alternate days, six 30-second exposures of either a control odor (ethyl alcohol) or a conditioning odor (amyl acetate). Sixty seconds following the presentation of the last conditioning odor of a session the animal was injected with apomorphine. Four control and 4 conditioning sessions were run. The dose of apomorphine was 6 mg/kg for the first 2 sessions and 12 mg/kg for the last 2. In similar experiments using taste rather than odor cues (see

Project Report M-LBEB-CN-9) conditioning was effective and led to a profound depression in drinking when the conditioned stimulus was present.

Major Findings: If odors become conditioned to apomorphine-produced illness, then a decrement in responding following presentation of the conditioning odor would be expected. In the present experiments, no significant interruptions in the on-going behaviors were observed: each animal oriented to the source of the odor and paused in responding for a few seconds when the odor was introduced, but these breaks in responding were brief and occurred equally often with either the safe or conditioning odor.

Significance to Biomedical Research and Program of the Institute. The present findings would be in accord with previous reports that olfaction has little or no cue value in mediating bait shyness or conditioned gustatory aversion. These and previous results (see Project Report M-LBEB-CN-9) further implicate taste as the primary conditioning factor in bait shyness and provides evidence for a functional separation of these two chemical senses in regard to conditioned aversions.

Proposed Course of the Project: Terminated.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-24
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neural substrate of nesting behavior of mouse
I. Corpus striatum

Previous Serial No.: None

Principal Investigators: Burton M. Slotnick, Robert Gelhard, and Paul D. MacLean

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.1
Other:	0.4

Project Description:

Objectives: The present project is part of an investigation testing the hypothesis that the corpus striatum plays a basic role in species typical behavior. Specifically, it tests the effects of bilateral lesions of the corpus striatum on nest-building behavior of the mouse, including the architectural structuring of the nest.

Methods Employed: Eight adult female albino mice were individually housed and given free access to thread as nesting material. In order to assure maximal nest-building behavior, each mouse received a subcutaneous implant of a 25 mg pellet of progesterone. Nests were weighed and scored on a four-point scale for architectural structure. Following a control period a electrocoagulation was made in the head of the caudate nucleus on one side in 4 mice. Twenty days later lesions were placed in the contralateral nucleus. Four mice served as sham-lesioned controls. Prior to termination of the experiment animals were also tested with 3-ply cotton string and with hemp twine.

Major Findings: The average nest weight during each stage of the experiment is given in the following table:

	Pre-surgical Control	After First Operation	After Second Operation
Caudate	9.7 g.	5.0 g.	2.2 g.
Control	11.7 g.	12.2 g.	7.9 g.

Unilateral caudate lesions resulted in an initial decrease in thread pulling, but each mouse showed a gradual recovery over the 20-day postoperative period. Following the second lesion these animals pulled in little or no thread, and only two animals showed some recovery of thread pulling during the remainder of the 40-day test period. There was a gradual reduction in thread-pulling by the control group that was not related to the sham-operation, but possibly reflected a reduction in action of the progesterone pellet. Control animals consistently built compact cup-shaped nests with the retreived thread. Striatal lesioned mice generally did not pull in enough thread to construct an adequate nest. However, when the two groups were given equal amounts of 3-ply cotton string or hemp twine there was no difference in the quality of nest construction.

The histological examination revealed that lesions were located in the dorso-lateral portion of the striatum. The lesions were smaller than intended and somewhat asymmetrical. Planimetric measurements will be made to assess the amount of striatal damage. On the basis of the estimated size of the lesion one would not have expected such a marked behavioral deficit.

Significance to Biomedical Research and Program of the Institute: After years of experimentation the functions of the corpus striatum, which forms a large and phylogenetically ancient part of the forebrain, have remained almost a complete enigma. The present study, together with the one described in Project M-LBEB-CN-1, suggests that it plays an important role in genetically constituted, species typical behavior.

Proposed Course of Project: To be continued.

Honors and Awards: None.

Publications: None.

Serial No. M-LBEB-CB-1
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Temporal Organization of Feeding Sequences and Sucking Behavior in Infant Dogs

Previous Serial Number: Same

Principal Investigator: Walter C. Stanley

Other Investigators: W. Edward Bacon, Stephen N. Lee, E. Faye Bacon, and Leslie D. Heiple

Cooperating Units: None

Man Years

Total:	1.64
Professional:	1.30
Other:	.34

Project Description:

Objectives: The aim of this project is to increase understanding of the temporal organization of neonatal behavior by studying the effects of environmental stimulation and internal bodily states on the component responses of feeding sequences in neonatal dogs. Prior research in this project has demonstrated such powerful effects on sucking of stimulus parameters, as nipple resiliency and length, stomach fullness, and repeated periods of daily food deprivations.

This year we continued to gather data on the ability of neonatal dogs to suck slowly and thereby obtain milk. Slow sucking conflicts with the neonate's natural tendency to suck rapidly, especially when milk is absent. It also conflicts with the tendency to suck in relatively fixed and rhythmic action patterns.

Methods Employed: Eleven newborn beagles were removed permanently from the dam and each maintained in one unit of the computer-controlled multiple-unit automated apparatus described in previous reports on this project. The effects on slowing, then speeding up of sucking, were studied as a function of computer-controlled DRL (Differential Reinforcement of Low rate of sucking), extinction (complete omission of milk), and FR (Fixed Ratio of sucks to milks) schedules of suck-contingent milk deliveries. Sucking behavior was objectively recorded in analog fashion by means of a Grass oscillograph and in digital fashion by quantizing sucks for recording as cumulative events on chart paper and as response events with correlated inter-response times on computer tape.

Major Findings: In our experimental situations, 0-50% of the neonatal puppies within a litter of purebred beagles have learned quickly to suck slowly and thus to obtain milk, while 50-100% of the puppies within a litter tend toward fast and maladaptive (nonmilk-obtaining) sucking. In this respect the neonatal puppy's sucking behavior is more like that of an adult pigeon pecking a key than of an adult rat, or other mammal, activating a manipulandum.

Significance to Biomedical Research and the Program of the Institute: Maladjustive behavior in children and human adults may be characterized by a lack of appropriate inhibition. This research on the temporal patterning of behavior contributes information on early experiential variables which may contribute to the slowing up of behavior (inhibition, suppression) and speeding up of behavior (excitation, facilitation, and hyperkinesis).

Proposed Course of the Project: Eight more beagle puppies will be run, four to study the slowing of sucking and four to study the speeding up of sucking. These data as well as previously collected data will be subjected to LINC-Computer graphic analyses and IBM-Computer statistical analyses, and the findings published in a series of reports on the temporal patterning of neonatal ingestive behavior. The project will be terminated next year or integrated with long-term developmental research (cf., Project No. M-LBEB-CB-10).

Honors and Awards: None

Publications:

Stanley, W. C.: Perspectives in behavior organization and development resulting from studies of feeding behavior in infant dogs. In Bosma, J. F. (Ed.): Third Symposium on Oral Sensation and Perception: The Mouth of the Infant. Springfield, Thomas, 1972, pp. 188-257.

Serial No. M-LBEB-CB-2
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Behavioral Effects of Response-Contingent Intragastric Versus Intraoral Milk Injection in Infant Beagle Dogs

Previous Serial Number: Same

Principal Investigator: Walter C. Stanley

Other Investigators: Rodney M. Sewell

Cooperating Units: Section on Endocrinology, Laboratory of Nutrition and Endocrinology, NIAMD (Margit Hamosh and Robert O. Scow)

Man Years

Total:	.45
Professional:	.15
Other:	.30

Project Description:

Objectives: In instrumental and operant conditioning, a reinforcer is an event that increases the frequency of behavior it has followed. For neonatal dogs, milk taken normally in the mouth is a powerful reinforcer. Is this reinforcing effect due to mouth stimulation, stomach stimulation, or some other correlated factor? Previous findings in the project indicated that direct intragastric milk injection via nasopharyngeal fistula is a moderately stable reinforcer with respect to sucking, an apparently unstable reinforcer with respect to button pushing, and devoid of reinforcing properties with respect to key lifting. Such data point to the preeminence of oral factors, both stimulus and response, in milk reinforcement. The project seeks direct evidence for the preeminence of oral factors in milk reinforcement by study of neonatal dogs surgically prepared for mouth stimulation without correlated gastric stimulation, and gastric stimulation without correlated mouth stimulation in the same animal. A surgically prepared dog has also been used to develop methods to study fat digestion to evaluate weight gain in esophagostomized puppies.

Methods Employed: A two-stage dual esophagostomy was carried out in five beagle dogs in the second week of life. In the first stage, a temporary nasopharyngeal intragastric cannula was inserted into place, the esophagus was exteriorized, and the skin closed under the exterior esophageal loop. Three days later, the esophagus was severed, and its cut ends allowed to retract to form "button-hole" shapes. The nasopharyngeal intragastric cannula was also removed and the puppy was ready for feeding or testing after insertion of appropriate catheters. A peristaltic pump was used to inject milk or other liquid into mouth (but with no correlated entry of milk into stomach) or into stomach (but with no correlated entry of milk into mouth).

Major Findings: We confirmed last year's finding that intra-oral injection of milk maintains sucking better than direct intragastric injection of milk. We also found that inflatable bulbs on catheters in both caudal and rostral fistulas could be highly aversive, producing distress vocalization, struggling, and complete cessation of sucking.

The milk injected into the mouth of one puppy was collected from the caudal end of the rostral esophageal fistula and analyzed by Drs. Hamosh and Scow to assess our esophagostomy preparation as a means of detecting presence of lingual lipase and its role in lipid digestion. The technique yielded negative results but suggested methodological improvements for further lipid analysis.

Significance to Biomedical Research and the Program of the Institute: The concept of reinforcement is one of the most widely generalized and conceptually integrating concepts relevant to the psychology, physiology, and biology of behavior. Knowledge of the anatomical locus of the action of food reinforcement in neonates should contribute to our understanding of learning and its relation to motivation and behavioral development. Knowledge of fat digestion could improve health and growth of neonatal dogs used in behavioral research.

Proposed Course of the Project: To study four to eight additional esophagostomized beagle puppies under varied conditions of stomach fullness, then terminate the project with publication of findings on locus of reinforcement and of fat digestion.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-3
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Schedule-Induced Maladaptive Adjunctive Behavior in Infant Beagle Dogs

Previous Serial Number: Same

Principal Investigator: Walter C. Stanley

Other Investigators: E. Faye Bacon

Cooperating Units: None

Man Years

Total:	.87
Professional:	.23
Other:	.64

Project Description:

Objectives: (1) To describe more accurately maladaptive behavioral states which occur in feeding situations and are characterized by the following: (a) sustained tongue position--pressed up against the roof of the mouth or pressed down on the floor of the mouth, and designated, respectively, tongue-on-the-roof-of-mouth (TOR) and tongue-on-the-floor-of-mouth (TOF); (b) generalized loss of normally directed and effective feeding movements involving mouth, head, trunk, and limbs; and (c) rigid and stylized body posturings and stereotypic and repetitive movements of mandible, head, trunk, and limbs. (2) To determine the relationships between specific oral stimulation and the tongue position responses in 4-8 day old and 15-17 day old infant dogs. (3) To determine the excitation correlated with tongue positions and body postures.

Methods Employed: For Objective 1, video taped pictures, photographs, and cumulative response records obtained in operant key lift situations when puppies display the maladaptive adjunctive behaviors were analyzed. Data on four puppies were intensively analyzed. For Objective 2, 30 younger and 30 older infant beagle puppies were repeatedly stimulated by placing in the mouth a milk-containing artificial nipple, a finger above the tongue, or a finger below the tongue. For Objective 3, suction-disc electrodes were tried with two puppies.

Major Findings: In the key lift situation, two of four puppies showed striking TOR/TOF behavior and it was possible to discern some interesting relationships: (1) A TOR/TOF usually did not occur until after the 10th or later insert precluding a simple explanation based on intense hunger; (2) A clear relationship exists between schedule of reinforcement and TOR/TOF although it is not a simple 1-to-1 relationship. For example, in the puppy showing the most maladaptive adjunctive behavior, the number and duration of TOR/TOF responses greatly decreased when the puppy was switched from a difficult to an easier reinforcement schedule. However, there was a delayed reaction. The decrease occurred not on the first easier session but on subsequent ones. In respect to Objective 2, intermittent stimulations to suck were followed by differential tongue position responses in both younger (4-8 day old) and older (15-17 day old) beagle puppies. When stimulation to suck was by finger placed below the tongue, the response was primarily sustained tongue position against the roof of the mouth. When stimulation to suck was by finger or milk-containing nipple placed normally above the tongue, the response was primarily sustained tongue position on the floor of the mouth. In respect to Objective 3, suction electrodes could not be held on the tongue of neonatal dogs long enough to provide valid lingual electromyography.

Significance to Biomedical Research and the Program of the Institute: Maladaptive, adjunctive, and dystonic tongue position responses and related posturings may provide an animal model for the study of tics, mannerisms, and dyskinesias in humans.

Proposed Course of the Project: Further research will be aimed at determining why only about 50% of our canine subjects show the maladaptive behavior in our operant conditioning situations.

Honors and Awards: None

Publications:

Stanley, W. C.: Induction of maladaptive behavior by experimental manipulation of feeding in infant dogs. In Bosma, J. F. (Ed.): Third Symposium on Oral Sensation and Perception: The Mouth of the Infant. Springfield, Thomas, 1972, pp. 400-419.

Serial No. M-LBEB-CB-4
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Capacity for Learning in Neonatal Dogs: Parametric Studies

Previous Serial Number: Same

Principal Investigator: Walter C. Stanley

Other Investigators: W. Edward Bacon

Cooperating Units: None

Man Years

Total:	.55
Professional:	.32
Other:	.23

Project Description:

Objectives: (1) To establish that neonatal dogs are capable of learning in classical, instrumental, and operant conditioning situations, with a variety of reinforcers. (2) To compare learning in neonatal dogs with that found in other neonatal mammals, including humans, as well as that found in adults.

Methods Employed: Four 5-day-old beagle dogs were run in the operant key lift apparatus following procedures described in last year's project report, Serial No. M-LBEB-CB-4.

Major Findings: Ordinarily, beagle puppies learn to perform the key lift response consistently before they are two weeks old. In the litter run this year, however, learning of the key lift response was unusually slow. One puppy attained a consistent level of response at 14 days of age, a second at 15 days, a third at 17 days, and a fourth at 18 days. Small body size of the puppies, height of key, difficulties in nipple latching all probably contributed to the slow learning.

Significance to Biomedical Research and the Program of the Institute: To understand early learning, we must know the conditions that preclude learning as well as the conditions that enhance it. This project is designed to contribute new knowledge concerning both the phylogeny and the ontogeny of learning processes.

Proposed Course of the Project: To continue analyzing data and to prepare reports for publication as follows: "Conditioning and Extinction of Avoidance and Escape Behavior in Neonatal Dogs," "Conditioning, Extinction, and Reconditioning of Operant Key-Lifting in Infant Dogs," and "Age Differences in the Effectiveness of Schedules of Operant Milk Reinforcement in Infant Dogs." The last mentioned study will require the running of eight additional neonatal dogs.

Honors and Awards: None

Publications:

Bacon, W. E., and Wong, I. G.: Reinforcement value of electrical brain stimulation in neonatal dogs. Developmental Psychobiology, 5: 195-200, 1972.

Bacon, W. E.: Acquisition of a temperature discrimination in neonatal dogs. Bull. Psych. Soc., 1: 65-67, 1973.

Bacon, W. E.: Aversive conditioning in neonatal kittens. J. Comp. Physiol. Psychol., in press.

Serial No. M-LBEB-CB-7
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Attachment Behavior in Beagle Dogs as a Function of Social Reinforcement

Previous Serial Number: Same

Principal Investigator: Walter C. Stanley

Other Investigators: Stephen C. Barker and W. Edward Bacon

Cooperating Units: None

Man Years

Total:	1.32
Professional:	.21
Other:	1.11

Project Description:

Objectives: Dogs form strong attachments to people. Experimental analyses of interspecific (dog-person) interactions have demonstrated that passive person is a reinforcer in a variety of breeds of dogs. Dogs increase their running speed if such behavior is followed by contact with passive person. If passive person is omitted, some breeds (basenjis and Shetland sheep dogs) lose much of their learned approach behavior; i.e., they display extinction of instrumentally conditioned approach behavior. In contrast, beagles persist strongly in their approach behavior long after person is omitted. The aim of the present project is to determine (1) why beagles persist so strongly in their learned social behavior, and (2) how a person reinforcer may be similar to, and different from, other reinforcers, both homeostatic (food) and nonhomeostatic (novel visual stimuli).

Methods Employed: In Experiment 1, 14 beagles were run, one trial per work day, in an L-alleyway, half with passive person in the goal area and half with goal area empty. All animals then received training with goal area empty. In Experiment 2, 10 beagles were run with person present throughout training and half with person introduced in the second half of training. In Experiment 3, five beagles were run with dry food as the reinforcer in the goal area and then with the goal area empty of food. In Experiment 4, 11 beagles were run, five trials per work day, in a 12-ft. long shuttle-box from which a dog was removed only after its day's running was finished. Half of each dog's trials were with passive person present in goal area and half with person absent. In Experiment 5, a shuttle-box is being used to determine how a dog's key-lifting and approach behavior vary when it is followed by visual exposure, to such potential reinforcers as the following: a seated passive person, a dummy of a passive person, another dog, and an empty goal area.

Group performance is analyzed in terms of speed of running measures obtained from clocks controlled automatically by photobeam circuits; individual performance, in terms of cumulative amount of behavior expressed as a function of cumulative time in test situations.

Major Findings: Experiments 1 and 2 indicated that L-alleyway running in beagles is enhanced by presence of person. However, some factor correlated with mere removal of dog from "the" goal area is also reinforcing since running behavior in dogs without the person also increased. In support of this notion persistence of running, in absence of person was markedly reduced in the shuttle-box (Experiment 4) where removal from apparatus occurred on only every fifth, rather than on every single, trial. Twelve beagle dogs are now being started in Experiment 5; no data are yet available.

Significance to Biomedical Research and the Program of the Institute: Study of sources of social reinforcement inherent in the interspecific dog-man interaction provides a paradigm for experiments ethically impossible in man and scientifically impossible in animals without strong attachment to man.

Proposed Course of the Project: To terminate this project with Experiment 5 or integrate the project with long-term studies of social behavior in intact litters of dogs. Preliminary reports of data were presented at the meeting of the Psychonomic Society in St. Louis and at the meeting of the Eastern Psychological Association in Washington, D. C. One journal report is nearing completion, a second is in the data analysis stage, and a third will be based on Experiment 5.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-8
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md. 20837

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Behavioral Mathematics and the Logic of Measurement

Previous Serial Number: Same

Principal Investigator: Walter C. Stanley

Other Investigators: None

Cooperating Units: None

Man Years

Total:	.26
Professional:	.11
Other:	.15

Project Description:

Objectives: To develop logic and mathematics directly relevant to the measurement of stable and changing behavior as it occurs in learning and other behavioral situations in order that amount of behavioral change may be validly compared from one response class to another in the same or different organisms.

Methods Employed: Most applications of mathematics to behavioral data represent empirical curve-fitting, statistical analyses, or models, the terms of which refer to inferred or assumed processes. The present effort differs from traditional approaches by starting from a theory of measurement rather than from a theory of behavior or learning. It also differs from traditional approaches by assuming that the relevant mathematics (number system and functions) must be abstracted, rather than inferred, from directly recorded and reinforced behavioral elements. Each behavioral element of a definable and recordable behavioral class is regarded as uniquely specifiable by the assignment of two numbers, one symbolizing cumulative amount of occurrence (c) of the behavioral elements, the other, time (t) of occurrence.

Major Findings: Relationships between assignment of numbers to behavior and scientific naming, classifying, and instancing were considerably clarified by showing, set-theoretically, that the number of potential classes in any collection of behavioral instances (responses) was always greater than the number of instances. Hypothetico-deductive, analogical, and mathematical (quantitative) explanations of behavior were evaluated. Mathematical explanation was found consistent with the objective dimensions of behavior as publicly observed and also with the dimensions of behavior privately experienced by the natural ("nonsubjective") scientist at work.

Significance to Biomedical Research and the Program of the Institute: The induction of a behavioral number system and mathematics from the study of behavior in experimental and naturalistic settings is propaedeutic to true measurement in the behavioral sciences.

Proposed Course of the Project: To continue work on the mathematics of behavior occurring in learning and other behavioral situations and to continue work in the development of a logic of behavioral measurement. Some aspects of the findings were incorporated in a comment entitled, "On Knowledgeable Behavior," submitted for publication to American Psychologist. A major explication of the findings appear in "Mind as Biobehavior," a manuscript prepared for publication in Science or similar journal. Extension of the mensurational system to quantification of developmental ethograms (precise inventories of all major behavioral patterns occurring in an animal during its individual development) is under consideration.

Honors and Awards: None

Publications: Incorporated into chapter entitled: Perspectives in behavior organization and development resulting from studies of feeding behavior in infant dogs. For references, see M-LBEB-CB-1.

Serial No. M-LBEB-CB-9
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Automated Apparatus for the Biopsychological Study of Behavior

Previous Serial Number: Same

Principal Investigators: Walter C. Stanley and Stephen N. Lee

Other Investigators: None

Cooperating Units: None

Man Years

Total:	.77
Professional:	.65
Other:	.12

Project Description:

Objectives: To design, construct, modify, and evaluate on-line computer-controlled and other apparatus suitable for the study of behavioral development in indoor (experimental) and outdoor (seminatural) situations.

Methods Employed: Assessment of apparatus and procedures via commercial and scientific literature and methodological studies in the Section, the apparatus and procedures combining the fields of comparative and experimental psychology, field ethology, and brain neurochemistry.

Major Findings: Programming and interfacing of the LINC (laboratory instrument computer) have been developed for the control of one pre-juvenile canine operant chamber, and will be tested with animals as soon as they are available.

Two adult canine operant chambers, previously controlled by BRS logic modules, are now also under the control of the LINC. Thus, control of more complex stimulus schedules can be achieved with ease and three-dimensional graphing of behavioral data is now possible after recording inter-response-time distributions on magnetic computer tape.

Improvements were made on video tape recording of dogs' behavior at the beginning of experiments and at times of behavioral change later in the experiments. A detailed pictorial study of behavior change is now possible.

Surgical techniques on esophagostomized puppies were video taped for future study, teaching, and reference.

Significance to Biomedical Research and the Program of the Institute: Improvement of highly specialized apparatus will: (1) permit more than one experiment to be conducted simultaneously by personnel available; (2) allow more flexibility and control in the objective recording of behavior; and (3) reduce greatly the time needed for data analysis.

Proposed Course of the Project: Heavy emphasis on apparatus for neonatal behavioral study is discontinued and placed instead on apparatus and procedures suitable for long term individual and group-life studies of behavioral development.

Honors and Awards: None

Publications: Incorporated in chapter cited under M-LBEB-CB-1.

Serial No. M-LBEB-CB-10
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Relationships Between Infant Learning Experiences and Adult Learning and Emotional Behavior

Previous Serial Number: None

Principal Investigator: Walter C. Stanley

Other Investigators: E. Faye Bacon, Gerson Grosfeld, Leslie D. Heiple, Stephen N. Lee, and Rodney M. Sewell

Cooperating Units: None

Man Years

Total:	.79
Professional:	.12
Other:	.67

Project Description:

Objectives: To determine how adult operant behavior (key-lifting) and correlated adjunctive and emotional behavior (drinking, licking, posturing, vocalization, etc.), are related to: (1) age when infant dogs are subjected to operant conditioning procedures; (2) schedules of milk-containing nipple insertions (reinforcements) present during infancy; and (3) expressions during infancy of both operant and adjunctive classes of behavior.

Methods Employed: In 1971-72, four beagle pups were run in an infant key lift apparatus. Two younger pups were started at 7 days of age, one receiving fixed ratio (FR) and the other fixed interval (FI) reinforcement. Two older infant pups were started at 15 days of age, again one receiving FR and one FI reinforcement training.

To assess the effects of this early experience on adult learning behavior, the pups were maintained as a litter in a common pen until they were 288 days old when they were subjected to daily operant training for about six months in an adult key-lift apparatus.

In 1972-73, the infant training procedure was replicated with a second 4-puppy litter. Additionall, a 4-puppy control litter was separated from the dam and laboratory-reared and fed just as the experimental litters had been, but without operant key-lift learning experiences as infants.

During infant training, key-lifts, nipple presentations, and time in apparatus were graphically and numerically automatically recorded. During adult training, key lifts, food presentations, licks on a water "fount," vocalizations, and time in apparatus were automatically recorded. Postures, licks at places other than the water fount, and fearfulness were noted on protocols.

Major Findings: During infancy, the older infant puppies learned faster than the younger infant puppies, although both age groups attained similar levels of performance. As young adults, younger-infant-trained puppies were deficient in starting to eat in the operant chamber, starting to key-lift, and in starting to respond to changed conditions of learning, but not in the rate of original learning nor in final level of learned key-lift performance. The second and third litters, now being run, will tell us if our initial major findings might be due to the differential infant (early vs. later) learning experiences, per se, or to some correlated factor such as earlier separation from mother, more prolonged feeding deprivation, or social dominance by the physically larger late learners over the physically smaller early learners.

Significance to Biomedical Research and the Program of the Institute: Human infants and children vary in the kinds of their early learning experiences. The present project will suggest what kinds of behavioral enhancements and/or deficits to look for in human adults with known early learning experiences.

Proposed Course of the Project: To finish running the eight beagle dogs now in operant training and run at least one litter of four puppies to assess further the possible role of dominance-submission variables.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-11
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Assessment of Methods of Electromyographic Recording from Neonatal Beagle Dogs

Previous Serial Number: None

Principal Investigator: W. Edward Bacon

Other Investigator: Stephen N. Lee

Cooperating Units: Oral Pharyngeal Development Section, Oral Medicine and Surgery Branch, National Institute of Dental Research (J. F. Bosma)

Man Years

Total:	.04
Professional:	.03
Other:	.01

Project Description:

Objectives: To determine the reliability and validity of electromyographic recording from neonatal dogs' tongues and neck muscles and thereby provide methods for assessing tonicity during adjunctive maladaptive states as described in M-LBEB-CB-3.

Methods Employed: Suction electrodes (borrowed from J. F. Bosma and modeled after lingual recording electrodes developed at the Haskins Laboratories by Katherine Harris and coworkers) proved impossible to maintain either on the tongue or at other places on the neonatal beagle puppy. A second beagle puppy, 6 days old, was prepared for electromyographic recording by shaving the hair from the dorsal and lateral surfaces of the neck and the dorsal surface of the base of the tail. Electrode paste was rubbed into the skin in the shaved areas to reduce skin resistance.

Three small-animal skin electrodes (Beckman) were attached by means of adhesive tape to the skin at the base of the tail (ground electrode) and to the skin over the belly of each longissimus capitis muscle. These muscles, when functioning together, elevate the head and, when acting singly, pull the head laterally.

Changes in bioelectric potential of the muscles were recorded on a Grass Polygraph (Model 7). Bioelectric potentials were fed to the preamplifier (Model 7P-3) with the following settings: calibration = 100 µv/cm.; 1/2 amp. low frequency at .3; function switch set at "amp". Paper speed was 25 mm/sec.

Major Findings: Results indicate that the electromyographic recordings were relatively free of artifacts due to electrode movement, etc. Changes in potential were greatest when the animal raised or moved its head to either side and changes were least when the puppy was in a quiescent state. Potential changes were intermediate when the animal was sucking either nutritively (Playtex feeder) or nonnutritively (finger). Occasionally, bursts of increased muscle activity would be reflected on the record while the animal was sucking. The bursts appeared to be correlated with pronounced stripping action on the nipple or finger. The technique described above seems adequate for recording changes in bioelectric potential in the larger muscle groups of the infant dog.

Significance to Biomedical Research and the Program of the Institute: The procedure can be used to evaluate tonicity in infant beagles during adjunctive maladaptive behavioral states. Along with appropriate neurophysiological and neurochemical alterations in brain loci, the technique could be used to assess the role of basal ganglia and cerebellar functions during the maladaptive behavioral states.

Proposed Course of the Project: Project is terminated.

Honors and Awards: None

Publications: To incorporate method description in other publications from the Section.

Serial No. M-LBEB-CB-12
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: General Activity as Function of Age, Food Deprivation, and Maturation of Internal Temperature Control in Infant Beagle Dogs

Previous Serial Number: None

Principal Investigator: Walter C. Stanley

Other Investigators: W. Edward Bacon, Gerson Grosfeld, Stephen N. Lee, and Leslie D. Heiple

Cooperating Units: None

Man Years

Total:	.40
Professional:	.15
Other:	.25

Project Description:

Objectives: In the first 21 days of life infant dogs change from essentially poikilothermic (having little internal bodily temperature control) to homeothermal animals (having internal physiological control of temperature). This project sought to determine how this physiological change in temperature control might be reflected in the general activity of infant dogs as expressed under differential conditions of ambient temperature and hunger.

Methods Employed: The activity of 24 2-8 day-old beagles was measured by placing each animal for five minutes in a circular cage on the surface of a multiple coil-generated electrical-force-field activity meter. Fine movements (heavy breathing) as well as gross bodily displacements were reflected in activity counts automatically recorded numerically on counters and graphically on cumulative event recorders. Half the 24 beagles were 9-11 hr. food-deprived and half 31-32 hr. food-deprived; and one third were tested in cold (65 degrees F), one third in warm (85 degrees F), and one third in hot (105 degrees F).

An additional 24 17-21 day-old beagles were similarly tested, then a third set of 24 puppies were tested to pin down what was happening between 16-18 days of age.

Major Findings: In the first week of life, general activity in neonatal beagles was a direct function of coldness and hunger with no interaction. At the end of the third week of life (19-21 days of age) puppies were relatively indifferent to cold and warm and to differences in hunger but were active when hot. Activity when hot apparently reflected the aversiveness of heat for an older puppy even though this same heat induced quiescence and sleep in week-old puppies. General activity in puppies 16-18 days of age represented a mixture of early and later effects of temperature and hunger but these data are still being analyzed.

Significance to Biomedical Research and the Program of the Institute: Contrary to introductory text-book treatments of motivation, general activity does not represent simple control by ambient temperature and level of hunger. Rather, the amount of activity is controlled only partly by such factors and partly by availability for occurrence of naturally stimulated escape vs. quiescent behaviors in particular test situations.

Proposed Course of the Project: The data gathering phase of the project is terminated.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-13
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immediate Adaptive and Maladaptive Consequences of Behavioral Requirements in Infant Learning Situations

Previous Serial Number: None

Principal Investigator: Walter C. Stanley

Other Investigators: Gerson Grosfeld

Cooperating Units: None

Man Years

Total:	.22
Professional:	.08
Other:	.14

Project Description:

Objectives: Artificial and natural learning situations to which mammalian infants may be exposed can have as their consequence renewed vigor or, in the extreme, death. For example, research in the Section on neonatal learning suggests that extreme care must be taken when learning requirements are made more stringent, otherwise the animal underfeeds and weakens. The purpose of this project is to: determine whether neonatal puppies maintain food intake or work output per unit time when they suck on artificial milk-containing nipples presented on: (1) predetermined fixed schedules; (2) a continuously available (demand) schedule; and (3) both operant (key lift) and consummatory (nipple-latch-and-suck)-dependent schedules.

Methods Employed: Work on Purpose 1, above, was held in abeyance this year. For Purpose 2, three puppies were placed overnight repeatedly in individual circular cages with 1-4 side-arm burettes with artificial nipples attached in each cage. For Purpose 3, two puppies each received an 11-hr. daily placement in the operant key-lift apparatus described in Project number M-LBEB-CB-10.

Major Findings: Overnight feedings, 17 hr. per night, with milk-containing nipples continuously available did not lead to appreciable weight gain in puppies in the first few days. But after about 4 days, puppies were gaining weight at the rate they would be with an excellent caretaking natural mother.

However, some of the puppies needed considerable help initially to learn to take milk from the artificial nipple. Data on key-lift puppies have not been analyzed yet, but preliminary inspection of records suggests that 11 hr. per day was not enough time for obtaining milk necessary for normal weight gain when multiple lifts of the key were required to make available 1 cc. of milk via nipple.

Significance to Biomedical Research and the Program of the Institute: Many interpretations of the effects of early experience on later behavior are based on speculation without factual support. This is especially true about the presumed effects of early learning. Extant scientific literature from this Section and elsewhere points, rather, to the extreme impermanence of early learning effects unless deleterious environmental situations are continuously present to adulthood.

Proposed Course of the Project: To hold the project in abeyance until additional personnel are available to continue it.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-14
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Passive Avoidance Learning in Neonatal Dogs

Previous Serial Number: None

Principal Investigators: Walter C. Stanley and Stephen C. Barker

Other Investigators: None

Cooperating Units: None

Man Years

Total:	.28
Professional:	.02
Other:	.26

Project Description:

Objectives: It is widely theorized that young animals are proficient in expressing behavior requiring excitation but deficient in behavior requiring inhibition. This theory is supported by some data gathered in the Section (Project no. M-LBEB-CB-5) but limited in generality by contradictory data on spaced sucking (Project no. M-LBEB-CB-1). The purpose of the present experiments is to examine behavioral suppression resulting from aversive stimulation in neonatal dogs. Previous research in the Section has shown that neonatal dogs are capable of active avoidance and escape learning, but no studies here or elsewhere have been done which clearly prove that neonatal dogs or similar mammals are capable of passive avoidance learning which requires suppression of the punished behavior.

Methods Employed: Seven purebred beagles, 2 days old at the start of training, were run in a one-way, cloth-to-plastic, shuttle box, four sessions per day, five trials per session. Puppies were allowed 2 min. to cross from cloth to plastic before removal from the apparatus. If they crossed, four puppies (passive avoidance) received 1 min. of cold air blast on the plastic side while three puppies (habituation controls) received 1 min. without cold air blast.

Major Findings: On the last day of training the four passive avoidance animals failed to cross 100%, 55%, 85%, and 90%, while the habituation control animals failed to cross on 0%, 0%, and 5% of the trials. These data indicate the presence of passive avoidance learning capacity in neonatal beagles.

Significance to Biomedical Research and the Program of the Institute: Work on neonatal passive avoidance learning is required to give a more complete picture of the learning capacity of neonatal mammals. In addition, the literature shows that various brain structures, notably the hippocampus, are differentially implicated in active vs. passive avoidance learning. Development of an efficient method for passive avoidance learning in neonates would permit future projects on developmental aspects of memory and brain-behavior correlations.

Proposed Course of the Project: To complete the study with about 10 more neonates, gathering data on extinction and reconditioning, then to publish the findings in a scientific report on neonatal passive avoidance learning.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-15
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effect of Recycling Progressive Ratios of Food Reinforcement on Operant Key-Lifting and Adjunctive Vocalization in Adult Beagle Dogs

Previous Serial Number: None

Principal Investigator: Walter C. Stanley

Other Investigators: E. Faye Bacon, Gerson Grosfeld, Rodney M. Sewell, and Leslie D. Heiple

Cooperating Units: None

Man Years

Total:	.21
Professional:	.07
Other:	.14

Project Description:

Objectives: Research of the Section has shown in neonatal beagles that a progressively increasing work requirement affected sucking behavior in only three of seven puppies. However, the work schedule affected vocalization in all seven subjects. The puppies vocalized more frequently during higher work requirements than during lower work requirements. The purpose of this project is to check the generality of these findings in adult beagle dogs.

Methods Employed: The subjects were two male beagles, 11 months old at the start and 16 months old at the end of daily training, usually seven days per week. Both dogs were tested in the adult operant chamber (Project no. M-LBEB-CB-10) with recycling increasing and decreasing progressive ratios of food reinforcement. For increasing ratios, the dog received its first food after 3 key lifts, its second food after 6, its third food after 9, etc., and its twentieth food after 60. The ratio requirement then recycled to 3 and increased by steps of 3 to 60 again, etc. for typically, 100 food reinforcements or five cycles in a daily session. For decreasing ratios, the order of ratios was reversed, i.e., 60, 57, 54, etc. to 3, then a recycle to 60, etc.

Major Findings: Details of data are still being analyzed but two striking effects were obtained. One effect was concerned with individual differences and one with vocalization. Neither dog's post food-reinforcement pause in key lifting was consistently sensitive to changing ratios. Rather, one dog (a vigorous vocalizer) showed marked breaks in key-lifting during higher work requirements, while the other dog (relatively quiet and phlegmatic) key lifted methodically and equally rapidly through the changing work requirements. The second striking effect was the marked sensitivity of vocalization to the changing work requirements in the more emotional dog. Greater vocalization was correlated with larger work requirements. The more phlegmatic dog barked only occasionally, and then only under highly disruptive circumstances, for example, under changed conditions during weekends.

Significance to Biomedical Research and the Program of the Institute: The data when related to the Section's previous findings on infant dogs, indicate that learning experiences in early infancy as indexed by vocalization may be generally more traumatic emotionally than similar learning experiences in adulthood.

Proposed Course of the Project: To finish analyzing the data and to run 2-6 additional animals.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-BS-2
1. Laboratory of Brain
Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Social velocity

Previous Serial Number: Same

Principal Investigator: John B. Calhoun

Other Investigator: Halsey M. Marsden

Cooperating Units: Office of the Director, IR, NINDS
(H.M. Marsden assigned to SOBS from NINDS)

Man Years
Total: 0.6
Professional: 0.2
Other: 0.4

Project Description:

Objectives: To explore how social velocity is modified by group size, individual social history, population history, and structure of the physical environment. Social velocity is defined as the relative degree of activity and alertness of an animal in those parts of the environment where social interactions are likely to occur.

Methods Employed: Mice and rats are housed in groups of 4 to 2000 in large highly structured environments. Individuals are marked to permit visual or automated recording of place and degree of activity. Seven major studies are included. They encompass four groups or populations of rats and twenty-one of mice. All observations have been "formated" on magnetic tape for computer analysis.

Major Findings: Current analyses have focused on 1) mice maintained in groups of 8, 16, or 32 throughout their lives; 2) mice of Study #122 (see M-LBEB-BS-4) assigned to groups of 12 according to four behavioral types that developed in the large overcrowded population described in M-LBEB-BS-3, and 3) mice, representative of the four behavioral types, that continued to live in the large overcrowded population. According to earlier theory and observation, members of a group become differentiated with respect to velocity such that in an optimum sized group of N_o individuals the velocity varies from 1.0 for the most integrated individual to $1/N_o$ for the most withdrawn one, with the difference in velocity between any two consecutively ranked individuals being a constant. As the group size increases, all individuals become less active (more socially withdrawn) and approach the velocity of that of the individual showing the least velocity in an optimum sized group.

The recent analysis of the longer term studies indicates that the findings on groups of greater than optimum size holds mainly for the earlier stages of social organization. At a group size twice the optimum, the behaviorally more normal animals show increased velocity, while the less normal ones become depressed, having a velocity comparable to that of the lowest individual in an optimum sized group. With a second doubling of group size, a quarter of animals rated most normal become markedly hyperactive; another quarter resemble the low velocity animal of the optimum sized group, while the remaining half have even a lower velocity. The last-mentioned animals become nearly immobile and engage in essentially no social interactions. Thus the consequences of increasing the group size above the optimum are that some individuals are forced to become so active as to increase their exposure to stress related mortality factors, while the majority of the group become so inactive and so uninvolved as to become, in a social sense, "non-mice." Obviously, a group or population could not increase more than four-fold the optimum size without some major reorganization, because a further reduction in velocity would be impossible.

The population reported in M-LBEB-BS-3 went through four doublings of the optimum population size. As there discussed, many individuals born after the second population doubling became fine-looking specimen (the so-called "beautiful ones") but after weaning rarely engaged in any social interactions. Both _in situ_ measures of velocity, as well as those made on small groups removed from the crowded setting, revealed that _no_ behavioral types with a history of prior overcrowding approached the degree of velocity characteristic of mice reared in optimum sized groups. Rather, the velocity of most animals was comparable to that of the lowest velocity individual in an optimum sized group. Furthermore, in all groups studied the more an individual's social velocity became suppressed, the more it withdrew physically into places where social interactions were less likely to be demanded; and the less it engaged in social interactions, the less capable it was in executing non-social behaviors such as nest building.

Significance to Bio-medical Research and the Program of the Institute: Crowding continues to become more acute in human society. The concept of social velocity developed in our studies of rodents has led to a methodology of measuring velocity that could readily be applied to humans. Such application would enable an evaluation of the degree of crowding and the likelihood of associated behavioral pathologies characterizing various patterns of social history and environmental settings. This knowledge can assist in recognizing pathological degrees of crowding, and thus enable an earlier search for remedial action.

Proposed Course of Project: Further analysis will continue with regard to social velocity based on our extensive data from several populations of mice and rats. In particular, focus will be placed on the details of individual history influencing social velocity or influenced by it.

Honors and Awards:

Participation in the panel on "Eco-epidemiology in preventive mental health programs" held in conjunction with the 1973 annual meeting of the American Psychiatric Association.

Presentation of an invited paper, "The Effect of Crowding on Social Velocity," at the 1973 meeting of the International Association of Social Psychiatry.

Publications: None.

Serial No. M-LBEB-BS-3
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The overliving of a mouse population

Previous Serial Number: Same

Principal Investigator: John B. Calhoun

Other Investigators: Halsey M. Marsden, K. Y. Ng

Cooperating Units:
1. Office of the Director, IR, NINDS (H. M. Marsden assigned to SOBS, LBEB, NIMH, from NINDS)
2. Section on Pharmacology, CBRS, NIMH

Man Years	
Total:	2.7
Professional:	0.7
Other:	2.0

Project Description:

Objectives: To determine the consequences of reducing death control factors to the point that more individuals survive than can be incorporated into the social organization of a population.

Methods Employed: Experimental and environmental designs remain as previously described 9M-P-C-(C)-37 for FY '70 as does also the selection of special groups for intensive study (M-LBEB-BS-3 for FY '72). During the current fiscal year observations emphasized place of residence, social affiliations, and characteristics at time of death of the last 250 survivors of a peak population of 2200 mice in Universe 25.

Major Findings: Prior to the birth of the last mouse to survive past weaning, and at a time when the population had declined to only about 100 individuals from the peak population size of 2200, and contained over 1000 individuals just approaching sexual maturity, the prediction was made that no more reproductive activity would take place and that the population would become extinct through gradual death of all members purely as a consequence of their aging. This forecast has proved correct; the last mouse died on January 8, 1973. This is the first time any population, natural or experimental, has been observed to become extinct due to failure of the members of its later generations to develop those sexual and aggressive behaviors essential for species survival. In summary: The colonizing four pair of mice were introduced into a large physical environment in which resources exceeded those necessary to support a population of 4,000 individuals and in which normal causes of death, except from aging, were markedly ameliorated. Under these circumstances the survival of most young led to a rapid increase in population; the total numbers doubled each 55 days. As animals matured they formed small social groups of 10 to 12 adults. Each group maintained dominance over a portion of physical space, its territory. When 14 such groups had formed, they controlled all desirable physical space, and effective expression of social roles could only take place within the context of the social space of group membership. Up until the filling of most physical space with organized social groups, young received excellent physical and social nurture. No role opportunities remained for these many maturing younger mice. Their attempts to secure such roles were repulsed by the territorial males in particular. Rejection of so many excess young, that were capable of filling social roles, led to a breakdown in capacity for territorial defense by the territorial males. Lactating females assummed this normal male role. Their aggression generalized to their own young, which were then ejected from the home site before they had developed the capacity for making affective bonds with associates. Further unfolding of normal social behavior was mechanically interrupted by intervention by other mice under the crowded conditions of life. During this time the rate of population increase declined to a doubling time of 145 days until it reached a peak of 2200 when mortality just balanced natality, momentary zero population growth. Most of the last 1000 born, the ones too early weaned by their mothers, never developed the capacity for socially relating. Not socially relating, they never engaged in mating activities; and not mating no young were born. By the time the population had declined to a lower density, compatible with possible resumption of breeding, all the mice had reached an age precluding normal reproduction. As reported in BS-4 and BS-6 for FY '72, a series of studies on such mice placed in comparable habitats at low densities at an age compatible with reproduction revealed that behavioral fragmentation and other behavioral pathologies characterized these mice to such an extent as to preclude population recovery even under more ideal conditions.

Terminal Behavior Types in a Crowded Population of Mice; Social Velocity and Interactions of the Sixteen Selected Groups in the Aging Population: To determine the degree that species typical behavior had been retained after the population had begun to decline, 16 groups of 12 mice each, 8 groups of females and 8 of males were intensively observed in situ. The following presents the results for one major variable, that of social velocity -- the capacity to remain active and behaviorally alert. Following analysis strategies developed in the small group studies, (see BS-2 and BS-4) a first approximation of group velocity scores (activity or inactivity at low or high status locations within the universe) were obtained for the male groups. As expected, aggressive males and males which utilize the ring stand and cup ranked one and two on this measure. All other male groups, including two "socially withdrawn" groups, had low velocity scores. A similar social differentiation of groups was apparent when scores based on the sum of social interactions (for a group) were ranked and plotted. Aggressive animals were moderately high, withdrawn ones were low, and there were two intermediate groups. Based on similar interaction totals the eight female groups showed a comparable social differentiation between selected behavioral types. Overall, the depression of social velocity scores indicates that the capacity for normal social behavior had been markedly impaired, although recognizable differentiation of behavioral types persisted.

Significance to Bio-medical Research and the Program of the Institute: If appropriate consideration is given to species specific behaviors, capacities, and settings of living, the insights from the present study can be generalized to other mammalian species including man: Where a social system fails to provide opportunity for role fulfillment by those individuals with adequate behavioral nurture, these individuals with the capacity to play meaningful social roles will attempt to secure roles, but most will be rejected by those individuals already filling the existing roles. Under those conditions where such newcomers exceed in number the established individuals, then the very process of rejecting so many others will disrupt the fabric of the existing social system with accompanying inappropriate assumption of role playing by the previously socially established individuals. With sufficient disruption of normal behavior, mothers and other older individuals fail to react appropriately to very young individuals. As a consequence these later born individuals miss the early opportunity to develop effective affective bonds with older individuals. One consequence of this loss of inter-generational bonding is too-early weaning. If such ejected individuals then experience a high rate of undifferentiated contact with associates, this "interruption of action cycles" prevents maturation of complex social behavior. Among mice such complex behaviors include courtship and aggression. Not mating, no young are produced, and this leads to population extinction. Man's most complex behaviors are conceptual. Therefore, to the extent that man experiences a history comparable to those of the mice in this study, it would be anticipated that the entertaining of complex ideas, and their implementation, would first be affected. We theorized that if crowding could so affect man, its first outward expression would be an increase in fertility since effective family planning requires an understanding of many complex social, economic, and ecological processes that lie beyond immediate personal experiencing, and where this understanding is missing, reproductive restraint will be reduced. Independently of our study this hypothesis was confirmed by Prof. Galle and associates of Vanderbuilt University utilizing demographic data from the city of Chicago. To their surprise, they found that, regardless of economic status, education, or ethnicity, fertility increased with crowding.

Proposed Course of Project: By October 1, 1973 the two year effort to structure the magnetic file data base for this and related studies on the social behavior of mice will have been completed. Most of FY '74 will be devoted to data analysis and writing. Very little effort will be expended during this fiscal year on the actual conduct of any experiments, relating to this or other projects. Publications listed below have been general overview ones. In the forthcoming year we will pursue a strategy of sequentially analyzing the details of the zoography, behavioral differentiation, and social organization and disruption expressed during the history of this and related populations.

Honors and Awards:

1. Invited to be the only non-English participant in the Symposium, Man in His Place, held in London during the summer of 1972 as the annual symposium organized and sponsored by the Royal Society of Medicine. See publication below, Death Squared.

2. Gave the first Pemberton Lecture before the College of Physicians of Philadelphia, April 4, 1973.

Publications:

Calhoun, J.B.: The population crisis leading to the compassionate revolution and environmental design. World J. of Psychosynthesis. 4 (6): 21-28, 1972.

Calhoun, J.B.: Plight of the Ik and Kaiadilt is seen as a chilling possible end for Man. Smithsonian. 3 (8): 25-33, 1972.

Calhoun, J.B.: Death squared: The explosive growth and demise of a mouse population. Proceedings of the Royal Society of Medicine. 66: 80-88, 1973.

Calhoun, J.B.: Revolution, tribalism, and the cheshire cat: Three paths from now. Technological Forecasting and Social Change. 4: 263-282, 1973.

Marsden, H.M.: Crowding and animal behavior. In Wohwill, J.F. and Carson, D.H. (Eds.): Behavioral Science and the Problems of Our Environment. American Psychological Association, 1972, pp. 5-14.

Ng, L.K.Y., Marsden, H.M., Colburn, R.W., and Thoa, N.B. Population density and social pathology in mice: Differences in catecholamine metabolism associated with differences in behavior. Brain Research (In Press).

Serial No. M-LBEB-BS-4
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Role of prior social experience in altering adaptive behavior.

Previous Serial No.: Same

Principal Investigator: Halsey M. Marsden

Other Investigators: None

Cooperating Units: Office of the Director, IR, NINDS (H.M. Marsden assigned to SOBS, NIMH from NINDS)

Man Years

Total:	0.3
Professional:	0.1
Other:	0.2

Project Description:

Objectives: To determine the influence of prior experience in complex socio-environmental settings on the ability of individuals to reconstitute, functionally, individual behavior and group organization in new settings devoid of the inhibiting or disruptive aspects or processes characteristic of the former environment.

Methods Employed: The subjects were mice from a population study described in Project No. M-LBEB-BS-3. Data collection, data processing, and techniques of observations adhered closely to methods that evolved from that Project.

1. Study #122 - Males undergo social withdrawal in mouse populations at high density in structured habitats. In the present study twelve males in each of six groups were introduced to six new, identically structured, habitats. The groups were as follows:

(1) Aggressors - Dominant, territorial males.

(2) Pooled withdrawn - Males living in large, all-male groups on the floor of the habitat.

(3) Solitary withdrawn - Isolated, single males living on platforms adjacent to food and water resources.

(4) "Beautiful mice" - Males living in small, all-male groups in nest boxes in excellent physical condition.

(5)&(6) An old and young sub-category for groups 2 and 4 completed the total of six.

The density in the new habitats was 3 mice per cell in a four-cell habitat, reduced from about 137/cell in the former 16-cell environment.

2. Study #124 - The 4 animals with the highest social velocity (high social status) from each of the 6 groups in Study #122 were reassembled in two new social groups of 12. Conversely the 4 animals with the lowest velocity were assembled in two additional groups. Two animals from each group of Study #122 that had "middle" social velocity scores comprised a 5th new group of 12. All groups were placed in clean, 4-cell habitats.

Major Findings:

1. Study #122 - None of the six groups formed a normal social structure although the basics of each were similar. The "pooled withdrawn" mice retained their characteristic behavior, feeding or resting on platforms in aggregates of up to eight animals. A strategy for preliminary, and later detailed analysis for this and other small group mouse studies has been implemented. The emphasis is on the physical environment (the mouse "universe") as its dynamics are reflected in the behavior and other characteristics of the resident small groups (four to 32 individuals). Information in four sub-areas of this zoography has been obtained:

(i) Residence before 1000 EST. Mice in this study were on a 12-hour bright-light, 12-hour dim-light cycle. Four surveys taken during the light (inactive) portion of the cycle revealed extreme rigidity and stability over time in the distribution of animals across cells in all six universes. The basic distribution across the four cells of each universe holding a group of 12 males was 9-1-1-1. This clearly separated each universe into one high density cell and three of low density. Three territorial mice were restricted one to each of the three low density cells. Eight withdrawn individuals and a single dominant resided in the high density fourth cell. Thus a basic and consistent differential use of space was established which could then be related to other characteristics of the animals known to be living in these respective cells.

(ii) Nests. Mice were provided with nesting material (paper strips) ad libitum. The territorial cells contained nests of much larger size and higher quality. Thus, high status cells have nests which are superior to those found in low status cells.

(iii) Social Velocity. Each universe had a basically similar and distinctive social structure. The three territorial males and the single dominant ranked 1-4 on a velocity scale based on both presence at high or low status locations within a cell and on actual behavior at those locations (moving or active behavior versus resting behavior, see Project No. M-LBEB-BS-2). In all cases the seven or eight other mice were withdrawn, showing very low and barely differentiated scores. The overall picture was a split within each group into a socially active and a socially inactive (or withdrawn) subgroup. In several universes, a fourth kind of animal emerged, a middle velocity individual, not dominant or territorial but not withdrawn, displaying a rather "creative" and varied use of space within cells.

(iv) Sex Behavior. Related closely to rankings based on velocity scores were the rankings based on frequency of social contacts with females (see Project No. M-LBEB-BS-6). Territorial males and dominants ranked 1-4 on the basis of frequency of social interaction with females, whereas withdrawn mice showed little or no response to the presence of females in their universe over a two-week period. Middle velocity individuals were again intermediate.

2. Study #124 - The second attempt to socially organize was a failure in all five reconstituted groups. There was no evidence of consistent territorial behavior by any animal. The animals appeared totally unable to cope with this second socioenvironmental challenge.

Significance to Biomedical Research and Program of the Institute. It is concluded that results of exposure to high-density living, resulting in social withdrawal and maladaptive behavior are, at least in part, irreversible in new environments devoid of the original influences of crowding. Persistence of behavioral pathology in new "ideal" settings when coupled with an inability to organize socially when challenged could have profound implications for mental health if the parallel is made with the human scene.

Proposed Course of the Project: Observations have been terminated. Analysis and reporting of data will continue. A new and important aspect of this project has emerged. The small, all male groups in the "experimental mouse universes" provide a relatively simple but powerful methodology, not only to test the effects of social experiences, but also the critical triune relationship between physical environment, social environment, and brain as it affects behavior. (See Project No. M-LBEB-CN-14) [Slotnick and Marsden].

Honors and Awards: Items pertaining to Dr. Marsden are as follows:

1) Invited Lecturer and Discussant: "Overliving in a Population of Mice." Environmental Studies Center's Institute in Environmental Management, Bowling Green State University, July 6-7, 1972.

2) Invited Chairman: Animal Behavior Society's Session on Physiology and Behavior, AAAS Meetings, Washington, D.C., December 30, 1972.

3) Invited with John B. Calhoun to prepare a paper, "The Effects of Crowding on Social Velocity," for presentation at the 1973 Meeting of the International Association for Social Psychiatry, Honolulu, Hawaii, May 4-17, 1973.

Publications: None

Serial No. M-LBEB-BS-8
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Environmental modification of individual behavior and social organization

Previous Serial No.: Same; continuation of M-LBEB-BS-7

Principal Investigator: John B. Calhoun

Other Investigators: Halsey M. Marsden, Leonard E. Olson, James S. Bryan, Harry Klopf

Cooperating Units:
1. Office of the Director, IR, NINDS (H.M. Marsden assigned to SOBS from NINDS)
2. Department of Physics, Virginia Polytechnical Institute (L.E. Olson maintains collaboration in this project while completing his doctorate there)
3. Section on Technical Development, NIMH

Man Years

Total:	2.5
Professional:	1.0
Other:	1.5

Project Description:

Objectives: These are threefold: (1) To develop theory for designing the physical environment in ways that will affect individual behavior and social organization sufficient to design environments that will permit investigation of individual and social behavior, as well as social system function. (2) To develop a technology capable of continuously recording the identity, place, direction of travel, and time of 500 rats free moving in complex designed environments. (3) To determine how three categories of environmental variables (i.e., pharmacological agents, social environment and physical environment) influence physiological function, behavioral capacity, and stability and complexity of social organization.

Methods Employed: (1) Our general theoretical model holds that all physical environments, that are supportive of social systems, essentially consist of compartments with avenues of communication between compartments, and that each type of compartment has structural, functional, and geographic uniqueness. The pilot environment consists of 13 compartments with 15 intercompartment linkages. It is bilaterally symmetrical with anterior dominance introduced by differential behavioral demands among compartments. (2) The present data acquisition system (DAS), developed in conjunction with Technical Development, NIMH, and the Biological Instrumentation Division, NIH, permits meeting the objective of recording the identity, place, direction of travel, and time for every rat in a population. See M-LBEB-BS-8 for FY'72 for other details. (3) So far we have conducted two studies in this setting. The first habitat containing 24 rats served as a "Project Proof" to test out the system. It included extensive visual observations to calibrate the system in the sense of determining the degrees of correlation of patterns of behavior that can only be visually observed with those longer, historical sequences recorded on the computer. In the second study a STAW (Socialization Training - Water) instrument placed in the two anterior compartments permitted sequential demands for securing water: a) free access; b) two rats must be present for either to get water; c) water is obtainable only if one rat is present. The first condition establishes a behavioral base line, the second "cools" the system, while the third "heats" it -- in analogy to gases.

Major Findings: What has pleased us most about the initial analyses is that the durations of merely being in the several compartments permits calculation of four probability functions that reflect basic settings of the central nervous system for each member of the population. For each behavioral state composed of the set of activities transpiring in any particular compartment these relate to a probability $\hat{p}$ of initiating the behavioral state, p' and p" which influence the termination of that state, and p_c which governs the probability of any two behavioral states becoming concatenated. In earlier studies with more primitive equipment, it was thought that such probabilities could only be determined by removing each subject from its social setting and isolating it for several days during the recording of behavioral sequences. For this reason, studies formerly subsumed under M-LBEB-BS-7 are now subsumed under M-LBEB-BS-8. Full restructuring of the raw data tapes have just been completed in a fashion that will make them most useful for analysis. The following non-substantive statement reflects more of the philosophy underlying current explorations of the data which are permitting fusion of perspectives from the fields of architecture, ecology, psychology, sociology, neurophysiology, and physics.

The methodology described above is capable of studying a general class of problems describable as: "an N-animal social system observed over time as a dynamic process of individual and group behaviors exhibiting both static and dynamic patterns collectively termed social structure." This process occurs in an environment which, in its elements and their relationships, is itself structured. In order to formulate a model of social structure in this context, we have found it necessary not only to utilize the formulations of the social and behavioral sciences, but to draw on methods and concepts from other disciplines, notably experimental particle physics. For instance, one of the most promising ways of expressing this complexity utilizes a propagator model. The propagator $P(x,x',t)$ gives in our case the probability that an animal will be discovered at position x' at time t given that he was at position x at time 0. Such propagators are studied in relativistic quantum mechanics by means of perturbation expansions (sums of infinite series of terms of decreasing magnitude where each term represents an interaction of increasing complexity) are symbolized by individual graphs (sub-network of events) called Feynman Diagrams. If the total scattering process (interaction with other animals and environmental structure) is dominated by a few processes, graphs representing these terms can be selected out for summation. We hope to be able to express both the effects of the network of connected settings and the complexity of the many simultaneous social interactions as dominating processes within this propagator formalism. The latter has been used by others successfully to model problems in genetics and ecology.

In a second, related approach, essentially derived from general systems theory, the totality of interactions between the animals are conceived as resource transactions across subsystem boundaries in the total social system. We are currently examining whether the propagator formalism allows a simpler expression of resource boundary behavior, including territory, dominance hierarchy maintenance, and the existence of relatively fixed sequences of passages in the environment. At this time we are examining this hitherto unavailable volume of data via a computer-driven cathode ray tube display, to search for new classes of patterns in the spatial motion of the group.

Significance to Biomedical Research and Program of the Institute. The system is a sophisticated and sensitive tool for research. For that part of our research which focuses on the promotion of mental health through an understanding of the biological and physical determinants of social organization it has provided otherwise unobtainable data on how animals "use" their environment and are affected by it. This tool provides knowledge about such basic concepts as territoriality, dominance-subordinance hierarchy, stereotypy or fixation in movement patterns, and the internal (central) programming of resource utilization. That part of biomedical research, which must depend strongly on utilizing animals as subjects, has now reached a stage of sophistication that it must move into the study of animals in oversimplified settings and life experiences to have maximum utility in their application to the human scene where individuals customarily do live in complex settings. Theory of environmental design, coupled with the technologies of automated observation, now makes it possible for the first time to engage in the type of experimental studies that simulate the human scene.

Proposed Course of the Project: During FY'74 we hope to develop the on-line capabilities which will permit elaborate stepwise alteration of contingencies resulting from the behavior of members of the population. When this is accomplished, and simple aspects of its utilization tested, the major components of the DAS will have largely been completed -- to the extent of permitting an extremely wide range of complicated studies. Since any such set of studies requires several years to complete, a good portion of the coming year will be spent defining what studies promise to be the most illuminating.

Honors and Awards: None

Publications:

Calhoun, J.B.: Disruption of behavioral states as a cause of aggression. Nebraska Symposia on Motivation, 1972: 183-260, 1973.

Serial No. M-LBEB-BS-9
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Population, space and mental health

Previous Serial Number: Same

Principal Investigator: John B. Calhoun

Other Investigators: None

Cooperating Units: None

Man Years
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: To develop philosophies of, and methodologies for, determining the impact of environmental and population changes on mental health.

Methods Employed: On the basis of insights gained from the research of this section, from the literature, and from discussions with interested parties, theoretical formulations are developed that might be useful for individuals and organizations within our society that must deal with the complex processes which influence mental health, societal stability, and cultural evolution.

Major Findings: The central trend of evolution is one of increasing capacity for awareness and responsiveness by individual elements or collections of them. As has been stated metaphorically, the evolutionary changes have involved fourteen "quantum" jumps in organization affecting information "metabolism." Each successive stage of organization is estimated to require only one-fourth the time for completion as did the prior stage. The first seven stages are regarded as involving the evolution of the biological brain from simple neural nets to the human brain. The second seven stages are viewed as involving the evolution of "social-prosthetic" brains encompassing ever larger networks of relationships among individuals and institutions, utilizing increasingly more sophisticated prostheses for storage and transformation of information. At the estimated rate, completion of the last two stages might be accomplished during the next 50 years. Thereafter one might visualize a situation in which each successive stage of increasing awareness and responsiveness would require a longer time than the prior stage. Contribution to promoting evolution requires development of new institutional processes, particularly those involving designed communication networks and information "metabolising" prostheses, which contribute to value change and the origin of new insights. Each of the items listed under Honors or Publications presents background to or suggestions for such institutional changes.

Significance to Bio-medical Research and the Program of the Institute: Rates of change, opportunity for resolution of value conflict, participation in communication, and fulfillment through adopting or creating insights, all affect mental well being. Harmonizing rate of change with capacity for response requires development of larger networks of communication capable of coping with complex problems. Since complex behavioral-social-environmental problems fall within the mission concern of the NIMH, more effective accomplishment of this mission could arise through establishing a metascientific organization as an extension of normal science organization, in a manner that facilitates coordinated effort from the recognition of problems suitable for research, through completion of the research, and the application of insights in the public domain.

Honors and Awards:

Continued to serve as a special consultant to the Committee on Preventive Psychiatry of the Group for the Advancement of Psychiatry. Served as assistant editor in the preparation of the final report of the committee entitled, "Humane Reproduction."

At the invitation of the Environmental Protection Agency prepared a policy background paper entitled, "Environmental Design Research and Monitoring from an Evolutionary Point of View."

At the invitation of an informal ad hoc discussion group consisting of policy makers in the private and public sectors gave a seminar entitled, "Metascientific Research."

Served as special editor of the Nov.-Dec. issue of the Journal of Environmental Health devoted to the topic of environmental behavior.

At the request of the National Science Foundation prepared a report, Prosthesis to Consensual and Creative Conceptualization, as a contribution to their effort to ascertain potential technological breakthroughs meeting human needs and national gain.

Publications:

Calhoun, J.B.: Values from an evolutionary perspective. J. of Environmental Health. 34(6): 578-583, 1972.

Calhoun, J.B. and Wheeler, G.G.: Environmental behavior: Origins and perspectives. J. of Environmental Health. 35(3): 220-225, 1972.

Calhoun, J.B.: Man of the environment. J. of Environmental Health. 35(3): 236-246, 1972.

Calhoun, J.B.: What sort of box? Man-environment Systems. 3(1), Section C-1: 30pp., 1973.

Calhoun, J.B.: Introductory remarks to the symposium: Environmental Design Research in the Social and Political Context. Proceedings of the Fourth Environmental Design Research Association Conference, Vol. 2, Dowden, Hutchinson & Ross, Publisher, 8pp., 1973.

Serial No. M-CM-DN-1
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The mechanism of action of thyroxine and its relation to cerebral metabolism

Previous Serial Number: Same

Principal Investigator: Louis Sokoloff, M.D.

Other Investigators: Patricia A. Roberts, Barbara A. Jant, Elaine E. Kaufman, Gilman D. Grave, Glenna G. Fitzgerald, Ellen G. Archer, John E. Kline and Esther L. Lewis

Cooperating Units: None

Man Years:

Total:	6.75
Professional:	2.75
Other:	4.00

Project Description:

Objectives: The ultimate goal of this project is the elucidation of the role of the thyroid hormone in the maturation and development of the brain and the influence of the hormone in the regulation of cerebral metabolic processes. A necessary intermediate objective is the determination of the molecular mechanisms of the physiological and biochemical actions of the thyroid hormone. Earlier work done previously in this project demonstrated a stimulating action of thyroid hormones on protein biosynthesis. The mechanism of action of thyroxine still remains the primary focus of the project.

Methods Employed: The effects of thyroid hormones on radioactive amino acid incorporation into protein are examined in cell-free protein synthesizing systems from various mammalian tissues, such as liver, brain, reticulocytes, etc. Studies are occasionally carried out *in vivo*, in which the uptake of amino acids into protein in various tissues is measured in the intact animal or the effects of experimentally induced thyroid disease on various metabolic activities are examined. A wide variety of enzyme purification and assay procedures are also employed. Enzymes are purified from brain or liver homogenates by detergent treatment, salt fractionation, gel adsorption and filtration, ion exchange chromatography, etc. Assays of enzyme activities utilize chemical analyses, spectrophotometric techniques, and Geiger-Mueller and liquid scintillation radioactivity measurements.

Major Findings: The major accomplishment of this project thus far is the demonstration for the first time that thyroxine stimulates protein biosynthesis and that this effect is probably primary to most of the generally recognized physiological actions of the thyroid hormones. Thyroxine, administered *in vivo* or *in vitro*, stimulates amino acid incorporation into protein in cell-free rat liver preparations. Conversely, hypothyroidism induced by surgical thyroidectomy, results in a reduction in the rate of amino acid incorporation into protein in similar preparations, indicating that the thyroid effect on protein biosynthesis is tonically active and physiological.

The biochemical mechanism of the thyroxine stimulation of protein biosynthesis has been studied in considerable detail in completely *in vitro* systems. These studies have been carried out over a number of years and have demonstrated that the thyroxine stimulation of protein synthesis is two-fold. There is a direct effect of the translational activity of ribosomes leading to accelerated completion and release of newly synthesized polypeptide chains followed, *in vivo* only, by an increased synthesis and accumulation of ribosomes, i.e., a transcriptional effect. The transcriptional effect is not a direct action of the hormone but a cellular response or compensation for some earlier effect. The early effect is the one most likely to lead to the elucidation of the primary mechanism of action of the thyroid hormones and is the one which has been of greatest interest to the Section.

The early translational effect has also, however, been found to be secondary to some earlier direct energy-dependent reaction between thyroxine and the mitochondrial components of the cell. Some still unidentified product of this reaction is found which acts on the ribosomes to stimulate their translational activity. Work has been continuing to isolate, purify, and identify this active factor. It is now known to be a small, dialyzable, heat-stable, acid-labile, anionic, organic, probably non-lipid substance. Continued work on it has increased the knowledge of its properties and narrowed the range of possibilities, but its exact identity still eludes us. This research has already led to the explanation of the change in sensitivity to thyroid hormones which occurs in brain during development. The brain responds to thyroid hormones in a manner similar to other organs during the period of its development and maturation. Once mature, however, it loses its responsivity to the hormones. This change can be seen in the effects of thyroid hormones on oxidative metabolism, protein synthesis, etc. One outcome of this project has been to show that the loss of sensitivity of the brain to thyroxine with development is the result of some change in the mitochondria so that they lose the ability to react with thyroxine and form the active factor responsible for the stimulation of protein synthesis.

Evidence has been accumulating that thryoid hormones may also have important effects on the transport of ions into and out of cells. Experiments have been carried out in this laboratory on the effects of thyroxine on the various microsomal ATPases in rat liver cells. Among them is a Na^+,K^+-stimulated ATPase which is intimately involved in the active transport of Na^+ and K^+ between cells and their external environment. Thyroxine has been found to inhibit the overall microsomal ATPase activity. Attempts to identify which of the various ATPases are affected have thus far shown no specificity. In intact, fresh rat liver microsomes, the Mg^{++}-, the Ca^{++}-. and the K^+,Na^+-stimulated ATPases are all inhibited by thyroxine. Qualitatively similar but quantitatively greater effects have been found with isolated liver plasma membranes and brain microsomes. These results suggest that thyroid hormones may also have a role in the regulation of intracellular ion concentrations. This would in turn have major effects on a vast variety of intracellular metabolic processes. In order to ascertain whether these effects found in vitro have physiological significance, the effects of experimental thyroid disease have been investigated. Hyperthyroidism leads in liver but not in mature brain to an enhancement of the microsomal ATPases, particularly the Ca^{2+}- and Mg^{2+}-activated species. These effects are in the opposite direction from those seen with the hormone in vitro. These opposing effects can be reconciled by the hypothesis that the direct effect of thyroid hormones is to inhibit the ion-dependent microsomal ATPases, but in vivo the cells compensate for this inhibition by producing more enzyme. This hypothesis can be evaluated by studies of the effects of thyroid hormones on the intracellular concentrations of the various ions. Such studies are currently being designed.

The thyroid hormones promote the structural, functional, and biochemical maturation of the brain. In addition to the studies of the mechanism of action of the thyroid hormones, the Section has also been studying the nature of the biochemical changes which occur in the brain with maturation. A prominent developmental change in the brain is a rise in the level of mitochondrial enzymes involved in oxygen consumption and energy metabolism of the brain. One of these, D-β-hydroxybutyric dehydrogenase (BDH), was found in this laboratory to rise with brain development just like other mitochondrial enzymes but, unlike these others, such as cytochrome oxidase and succinoxidase, to decline again gradually following weaning. Evidence has been obtained that the changes in the level of this enzyme in brain are, at least in part, related to nutritional factors. It has also been found recently that hyperthyroidism shifts the developmental pattern in this enzyme to an earlier age (e.g., it causes a more rapid and earlier rise in the enzyme level in the brain, an earlier achievement of its peak level, and an earlier decline). Hypothyroidism would be expected to shift it in the other direction, but these studies have not yet been carried out. In the course of these studies an interesting observation was made which might be a valuable model for the elucidation of the mechanism of action of thyroxine in mitochondria. Physiologically active thyroid hormones and their analogues were found in vitro to inhibit BDH activity at extremely low concentrations. The inhibition was found to represent a competitive inhibition by the hormone of the binding of the normal substrates to the enzyme. The enzyme is a membrane-bound enzyme in mitochondria. After considerable effort it has been solubilized and partially purified several-fold from both brain and liver. The purified enzyme is to be used to study the mechanisms of the thyroxine-inhibition as well as the mechanisms of the regulation of the enzyme's activity through all its changes during postnatal maturation of the brain. It has already been found with the partially purified enzyme that the enzyme requires a phospholipid cofactor normally present in mitochondria for its activity. This cofactor has been identified as a species of lecithin which can be partially substituted for *in vitro* by commercial beef heart or egg lecithin. The natural cofactor has been partly purified, and progress has been made to identify the nature of its fatty acid composition.

Significance to Bio-medical Research and the Program of the Institute: A unique feature of the cerebral metabolism is its apparent lack of response to high circulating levels of thyroid hormone. An understanding of the basis of this unique behavior may reveal information concerning the metabolism of the brain in health and disease. Also, the mechanism of action of thyroxine has been under investigation for many decades, but thus far a satisfactory explanation of the mechanism by which it increases metabolic rate, stimulates metamorphosis and growth, or causes the many disturbances in body physiology and biochemistry in thyroid disease has eluded investigators. The solution of this problem could then lead to fundamental advances not only in the understanding of how this important hormone regulates bodily functions but also of the nature of bodily processes on which it acts. The clarification of the relationship between its mechanism of action and its effects on development of the brain might also lead to a better understanding of the many biochemical processes underlying the maturation of the brain.

Proposed Course of Project: The project will be continued essentially on the course already indicated. The major avenues to be pursued are: (1) the attempts to isolate, purify, and identify the product of the thyroxine-mitochondrial reaction responsible for the stimulation of protein biosynthesis; (2) further studies on the elucidation of the mechanism of the stimulation of the protein biosynthetic processes; (3) the further study of the thyroxine inhibition of microsomal ATPase; (4) further studies of the biochemical differences between mature and immature brain and the role of thyroxine in the biochemical changes leading to maturation and development of the brain; (5) purification of brain D-β-hydroxybutyric dehydrogenase; and (6) studies of the mechanism of the inhibition of D-β-hydroxybutyric dehydrogenase by thyroxine.

Honors and Awards: None

Publications:

Sokoloff, L., and Raskin, N. H.: Chapter 96. Thyroid Disorders. In Shy, M., Appel, S., and Goldensohn, E. (Eds.). The Cellular and Molecular Basis of Neurological Disease. New York, Lea & Febiger, 1969 (in press).

Sokoloff, L.: The effects of thyroid hormones on protein synthesis. In Methods in Investigative and Diagnostic Endocrinology. North-Holland Publishing Co., Amsterdam, The Netherlands, 1972, pp. 148-171.

Grave, G. D., Satterthwaite, S., Kennedy, C. and Sokoloff, L.: Accelerated postnatal development of D(-)-β-hydroxybutyrate dehydrogenase (EC.1.1.1.30) activity in the brain in hypothyroidism. J. Neurochem. 20:495-502, 1973.

Sokoloff, L., and Kennedy, C.: Effects of Thyroid Hormone on Brain Development. In Biology of Brain Dysfunction. New York, Plenum Publishing Corporation, 1972 (in press).

Sokoloff, L.: Metabolism of Ketone Bodies by the Brain. In Annual Review of Medicine, 1973 (in press).

Sokoloff, L.: Prefatory Comments on Regulatory Biochemistry of Neural Tissues. Introduction for chapter in The Neurosciences - Third Study Program. MIT Press, Cambridge, Mass, 1973 (in press).

Sokoloff, L.: Changes in Enzyme Activities in Neural Tissues with Maturation and Development of the Nervous System. In The Neurosciences - Third Study Program. MIT Press, Cambridge, Mass. 1973 (in press).

Fellous, A., Francon, J., Nunez, J., and Sokoloff, L.: Protein synthesis by highly aggregated and purified polysomes from young and adult rat brain. J. Neurochem. (in press).

Serial No. M-CM-DN-2
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on regional cerebral circulation and metabolism

Previous Serial Number: Same

Principal Investigator: Charles Kennedy, M.D.

Other Investigators: Louis Sokoloff, Michel H. Des Rosiers, Jane W. Jehle, John E. Kline and Esther L. Lewis

Cooperating Units: Martin Reivich, M.D., Associate Professor, Department of Neurology
University of Pennsylvania
Philadelphia, Pennsylvania

Clifford S. Patlak, Ph.D., Theoretical Statistics and Mathematics Branch, NIMH

Man Years:

Total:	3.00
Professional:	1.50
Other:	1.50

Project Description:

Objectives:

1. To elucidate the patterns of development of circulation and energy metabolism in various structural components of the developing mammalian brain.

2. To quantify the effects of hyperoxia on nucleic acid synthesis and cell division in the developing brain.

3. To determine whether diphenylhydantoin affects the regional circulation of the brain when given in doses known to increase the seizure threshhold.

4. To continue the development of a method for measurement of regional glucose utilization of the brain *in vivo*.

5. To determine the effect of α-aminobutyric acid (GABA) on cerebral blood flow.

Methods Employed:

1. Regional circulation is measured by the autoradiographic ^{14}C-antipyrine technique developed in this laboratory.

2. Nucleic acid contents of brain tissue are measured by the Santen and Agranoff modification of the original Schmidt-Tannhauser procedure.

3. The method for regional glucose utilization is one which has been under theoretical development in this laboratory for several years. This is an autoradiographic procedure in which the non-metabolized analogue of glucose, 2-deoxyglucose is used as a radioactive tracer (^{14}C).

Major Findings:

1. Previously it had been shown that exposure of newborn rats to 70-80% oxygen for nine days resulted in an impairment to brain growth. This was due to a reduction in the rate of cell division. Because the effect observed was relatively small (7%), it was decided to repeat these studies confining attention to the cerebellum, a portion of the rat brain in which cell division is particularly prominent in the immediate postnatal period. It is hoped thereby not only to confirm the initial observation but to note whether the magnitude of the effect may be greater as might be predicted. This work is still in progress.

2. Studies of the effect of diphenylhydantoin (DPH) on local cerebral blood flow in the rat have been extended. A year

ago a series had been completed on two extremes of plasma concentration: one (< 10 μg/ml) lower than that known to be effective in preventing seizures and the other (30-50 μg/ml) which is toxic. No effect had been found at the low levels whereas in toxic dosage there had been a variable effect: some structures had values for blood flow higher than those in control animals; other structures had values which were below control levels. It was difficult to draw meaningful conclusions from the findings in the animals whose DPH levels in blood were toxic because there was a concomitant depression of respiration and, consequently, an elevation in arterial carbon dioxide tension (pCO_2). Such an elevation of pCO_2 is known to cause an increase in cerebral blood flow. Plasma levels of DPH of 20 μg/ml were found to be without effect on respiration, i.e., blood gases were the same in control and experimental animals. Under these conditions DPH was found to cause a reduction in values for local blood flow in all 21 structures of the brain where measurements were made. The greatest per cent changes were in the inferior colliculus (24.7%), auditory cortex (22.1%), superior olive (21.6%), and pontine gray matter (19.6%).

The question then arose as to whether this generalized effect of DPH on cerebral blood flow was a direct action on the cerebral vessels or whether it could be ascribed to an effect secondary to an altered metabolic rate. The latter consideration was plausible because cerebral blood flow has been shown to be adjusted to local metabolic demand and the studies had been carried out under conditions in which the DPH was not only in the blood, but was in brain tissue in comparable concentrations. Therefore a group of studies were carried out in which the DPH was primarily confined to the vascular compartment: e.g., by making the blood flow determination immediately following a DPH infusion. Under such conditions DPH-infused animals were found to have slight increments (5-15%) in cerebral blood flow compared to values in control animals.

It was concluded that DPH, when given in non-toxic doses as used to prevent seizure activity, results in a variable reduction in cerebral blood flow. This effect is probably secondary to a reduction in the metabolic rate. When the agent (in the same dose) is confined to the vascular compartment, there results a slight increase in blood flow.

3. The method for measuring local glucose utilization in the component structures of the brain in vivo is approaching the final stages of development.

This method is based upon a model which assumes that ^{14}C-deoxyglucose can be used as a tracer for glucose uptake by tissues. The fact that this substance is transported from blood to brain in a manner almost identical to glucose, yet remains unchanged after conversion to deoxyglucose phosphate means that this analogue accumulates in the brain at a rate equal to glucose utilization. In the previous year experiments had been carried out in rats and cats which indicated that a pool of free glucose in the brain is of sufficient size to cause an over-estimation of values. In the current year an estimate of the size of this pool has been made. For this purpose, another glucose analogue, 3-O-methylglucose, has been employed. It shares with glucose and deoxyglucose the same transport system for exchange between blood and brain, but differs from these by remaining chemically unchanged in the tissues. Thus its distribution in brain following an intravenous infusion can be considered to reflect that of free glucose. In a series of experiments in rats rate constants for the exchange of ^{14}C-labelled 3-O-methylglucose between blood and brain were calculated and its relative concentration in brain and blood were measured at equilibrium. This permitted the experiments for measuring local glucose consumption to be designed so as to minimize the error caused by the pool of free glucose and a correction to be made for the small remaining error. Values obtained to date are close to those expected from measurements made of overall glucose utilization in the brain. It remains now to test the method under conditions which are known to affect blood flow only and to demonstrate unchanged glucose utilization under such conditions. Also experiments will be planned where the conditions are such as to alter cerebral metabolic rate.

Significance to Biomedical Research and the Program of the Institute: The vast majority of individuals with defective mental development (mental retardation, minimal cerebral dysfunction) are considered to have suffered some derangement of normal metabolic processes during the period of most rapid brain growth, i.e., just before and just after birth. Recent studies, both experimental and those involving children, confirm the evidence that there is a short period of brain growth when the demand for substrate for energy and growth is such that deficiencies result in irreversible deficit. The studies carried out in this laboratory are an effort to measure aspects of normal energy metabolism and the interdependence of these on the cerebral circulation. Our earlier work has shown that the developing cerebral vasculature responds by marked vasoconstriction when oxygen is present in excess. With the development of a method for measuring local glucose utilization, it is proposed to determine whether or not increases in oxygen tension in the blood may limit the supply of glucose, an essential substrate for brain growth and function. The outcome of such studies may have direct bearing on current medical practices in the care of the human newborn, especially the prematurely born, which are prone to sub-optional mental development.

At the other end of the age spectrum, normal mental function is also known to be impaired by defective function of the cerebral circulation. The study of the effect of diphenylhydantoin on cerebral blood flow is timely in the light of recent reports suggesting its administration leads to a reduction of episodes of cerebrovascular occlusion. Similarly, the study of vasoactive agents, as it may elucidate the brain's normal control mechanisms for regulating cerebral blood flow, may prove of great importance in the prevention and control of cerebral vascular disease and its consequences to mental function.

The successful development of the method for local glucose utilization in brain has particular significance. For years there has been a hypothesis that blood flow to various regions of the brain is parsimoniously regulated according to the metabolic demand. The method now demonstrates directly and unequivocally that the hypothesis is indeed correct, and as a result much greater significance can now be attributed to the body of literature reporting on changes in local blood in the brain under a variety of physiologic conditions. One example is our own work on "Changes in Blood Flow in the Component Structures of the Dog Brain during Postnatal Maturation" (see Publications). The method opens new important areas of investigation of interest to workers in other disciplines such as neuropharmacology, neurophysiology and neuroendocrinology. It will provide an extremely useful tool in establishing the loci and neuronal centers involved in such functional activity as sleep, specific motor and sensory function, and various affective states, to mention only a few. It also will be important in establishing the locus of action of drugs. It will provide a means of mapping the sequence of brain centers which are activated following electrical stimulation. This will be particularly useful for the study of epilepsy in animal models.

Proposed Course of Project: With the finalization of the development of the method for local glucose utilization in brain we will return to the problems of the energy requirements of the brain during development. In particular we will focus attention on the brain's ability to employ substrates other than glucose during maturation, especially D(–)-β-hydroxybutyrate, and the implications of this supplemental energy source to normal brain maturation. The effect of oxygen will also be examined to determine whether its known action in reducing nucleic acid synthesis may be related to a limited supply of glucose.

Honors and Awards: None

Publications:

Kennedy, C., Grave, G. D., Jehle, J. W. and Sokoloff, L.: Changes in blood flow in the component structures of the dog brain during postnatal maturation. J. Neurochem. 19:2423-2433, 1972.

Sokoloff, L.: Circulation and energy metabolism of the brain. In Albers, R. W., Siegel, G. J., Katzman, R. and Agranoff, B. W. (Eds.). Basic Neurochemistry. Boston, Little, Brown and Company, 1972, pp. 299-325.

Sokoloff, L., Grave, G. D., Jehle, J. W. and Kennedy, C.: Postnatal development of the local cerebral blood flow in the dog. In Fieschi, C. (Ed.). Cerebral Blood Flow and Intracranial Pressure. Proc. 5th int Symp., Roma-Siena 1971, part I. S. Karger, New York/Basel, 1972, pp. 269-273.

Sokoloff, L., Grave, G. D., Jehle, J. W. and Kennedy, C.: Postnatal development of the local cerebral blood flow in the dog. *Europ. Neurol*. 6:269-273, 1972.

Kennedy, C., Grave, G. D. and Sokoloff, L.: Alterations of local cerebral blood flow due to exposure of newborn puppies to 80-90% oxygen. In Fieschi, C. (Ed.), *Cerebral Blood Flow and Intracranial Pressure*. Proc. 5th int. Symp., Roma-Siena, 1971, part I. S. Karger, New York/Basel, 1972, pp. 137-140.

Kennedy, C., Grave, G. D. and Sokoloff, L.: Alterations of local cerebral blood flow due to exposure of newborn puppies to 80-90% oxygen. *Europ. Neurol*. 6:137-140, 1972.

Des Rosiers, M. H., Grave, G. D., Kupferberg, H. J. and Kennedy, C.: The effect of diphenylhydantoin on local cerebral blood flow. Proc. 6th Int. Symp. on Cerebral Circulation and Metabolism, Philadelphia. Springer-Verlag, New York, 1973 (in press).

Serial No. M-CM-DN-3
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical bases of alcohol addiction

Previous Serial Number: M-CS-CM-11

Principal Investigator: Louis Sokoloff, M.D.

Cooperating Units: Neil H. Raskin, M.D., Department of Neurology, University of California Medical Center, San Francisco, Calif.

Other Investigators: None

Man Years:

Total:	0.25
Professional:	0.25
Other:	0

Project Description:

Objectives: This project is directed at the acquisition of knowledge of the biochemical changes associated with chronic alcohol ingestion and the integration of such knowledge into a framework which it is hoped will provide a basis for the understanding of the mechanisms underlying alcohol addiction and the withdrawal effects.

Methods Employed: The enzymes associated with ethanol metabolism and that of its products are assayed in brain, liver, and other tissues of normal rats and rats fed various amounts of ethanol for various periods. Evidence of enzyme inductions or repression by alcohol will be sought. Any changes in tissue enzyme levels will be examined for possible effects on the metabolism of other important metabolites on which these enzymes could conceivably act. In parallel studies purified enzymes of the alcohol metabolic pathway will be studied for substrate specificity and their effects on selected metabolites known to be important in the functional activity of the nervous system. Classical methods of enzymology will be employed.

Major Findings: This project is being worked on in collaboration with Dr. Neil H. Raskin, a previous Research Associate in this Section, and presently an Assistant Professor in the Department of Neurology, University of California Medical Center, San Francisco, California. This project had previously demonstrated by means of a new assay technique the presence of alcohol dehydrogenase, the enzyme responsible for the metabolism of ethanol, in the brain. Previous efforts by other investigators to find the enzyme in brain had been unsuccessful because of inadequately sensitive and specific methods. The kinetic properties and the responsivity to specific inhibitors of the brain enzyme have been examined and compared to those of the liver enzyme and found to be almost identical. A tissue survey of the alcohol dehydrogenase has revealed its presence in all tissues which exhibit pathological changes with chronic excessive alcohol ingestion. In studies in rats it was found that the brain enzyme activity uses about 50% with chronic alcohol ingestion. This is in contrast to the liver enzyme which remains unchanged. This is the first evidence of a specific enzyme change in the brain with alcohol intake and suggests a possible relationship to the development of tolerance to ethanol and/or a biochemical basis for the development of CNS symptoms following withdrawal from alcohol after prolonged ingestion. Current studies are underway to determine if the enzyme change in brain is associated with behavioral changes. Preliminary results suggest that the rise in brain alcohol dehydrogenase activity is paralleled by an increased tolerance for alcohol, but no evidence of addiction or alcohol-withdrawal symptoms has been obtained.

Significance to Bio-medical Research and the Program of the Institute: Numerous drugs and chemical agents cause addiction which results in withdrawal symptoms when their use is discontinued. Alcohol is one of the most common. Although it is clear that some biochemical change must have occurred, none yet has been found which could satisfactorily explain the phenomenon. Elucidation of the biochemical mechanism of alcohol addiction might not only serve as a model for other types of addiction but might also lead to a more rational approach to therapy.

Proposed Course of Project: Studies will be continued to determine whether the changes in brain alcohol dehydrogenase activity with chronic alcohol intake are associated with pathological, biochemical and behavioral changes. The brain alcohol dehydrogenase will also be purified and its substrate specificity determined with particular emphasis on normally present brain metabolites which might be critical for normal cerebral metabolism and function. This project has been inactive during the past year because of lack of personnel to work on it, but a Visiting Scientist who will enter on duty in October, 1973, will reactivate it.

Honors and Awards: None

Publications: None

Serial No. M-CM-DN-4
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Regulation of protein synthesis in the brain

Previous Serial Number: Same

Principal Investigator: Michael J. Schmidt, Ph.D.

Other Investigators: Louis Sokoloff, M.D.

Cooperating Units: None

Man Years:

Total:	1.25
Professional:	1.25
Other:	0

Project Description:

Objectives: One of the most prominent biochemical changes occurring in the brain during maturation and development is a change in the rate of protein synthesis. Earlier work in this and other laboratories has demonstrated in the rat that cerebral protein synthesis proceeds rapidly in the early postnatal period but declines markedly as maturation is achieved. The initial objective of this project is the elucidation of the control mechanisms responsible for this fundamental biochemical change with maturation.

Methods Employed: Protein synthesis is measured in cell-free preparations from brains of rats of various ages from birth to maturity. The soluble enzymes and the ribosomes, which comprise the cellular biochemical machinery for protein biosynthesis, are separated and purified by a variety of ultracentrifugation techniques and by isolectric precipitation methods. These are recombined in reaction mixtures containing the necessary energy sources, cofactors, and radioactive amino acids to permit the process of protein synthesis to proceed under optimal or test conditions. The direct effects of cyclic AMP and cyclic GMP on brain protein synthesis are examined under optimal conditions which have been established in previous experiments. The influence of a cyclic AMP-dependent enzyme, protein kinase, on the process of protein synthesis is also under investigation. The protein kinase enzyme is purified from rat brain by isoelectric precipitation, salt fractionation and column chromatography. Ribosomes isolated from the brains of rats at various ages are characterized by sucrose gradient ultracentrifugation and UV absorbancy. Purified ribosomes are incubated in the presence of cyclic AMP, the purified cyclic AMP-dependent protein kinase, and ^{32}P-ATP and the ability of these ribosomes to be phosphorylated is determined. Both endogenous protein kinase activity and phosphate acceptor activity of the various cellular fractions involved in protein biosynthesis are examined as a function of age. Particular attention is being given to the ribosomes since these cellular components are suspected to change functionally during the development of the brain. Now that we have established the optimal purification procedures and assay conditions for cyclic AMP-stimulated ribosomal phosphorylation and for protein synthesis it will be possible to examine under tightly controlled conditions the effects of phosphorylation of ribosomal protein on brain protein biosynthesis. These studies are currently in progress.

Major Findings: Previous work in this laboratory demonstrated that protein synthesis *in vitro* in cell-free preparations from rat brain declined about 75% between the ages of 15 days and 45-60 days. During this interval the major part of postnatal development and maturation of the brain occurs. These *in vitro* data are in close agreement with the results obtained by others *in vivo*, but the *in vitro* methods permit more definitive studies of the mechanism of the change. Other laboratories since have demonstrated that there is a progressive decline in protein synthesis in the brain with maturation and have implicated functional changes in the ribosomes as being, at least partly if not completely, responsible for the change.

The principal investigator has previously found an almost inverse pattern of change of the cyclic AMP system in developing rat brain. This system is relatively inactive at birth and then rises to the adult level during maturation of the brain. Cyclic AMP is known to stimulate the activity of a protein kinase, an enzyme(s) which catalyses the phosphorylation of many proteins. The question has, therefore, been raised whether the cyclic AMP-stimulated protein kinase may cause phosphorylation of proteins of the protein synthesizing system and thus modify their activity in protein synthesis. In particular, the hypothesis being tested is that the development of the cyclic AMP system during maturation of the brain leads to increased phosphorylation of ribosomal structural or functional proteins, thus causing lower ribosomal activity in protein synthesis with maturation.

The initial results have demonstrated that microsomes of brain contain protein kinase activity which is stimulated by cyclic AMP, but no differences were observed with age of the animal. The development of this enzyme in brain was not altered by early administration of thyroid hormones which are known to accelerate the development of a number of other neurophysiological and biochemical events in the brain.

With a more refined assay system it has been found that ribosomal proteins are fully capable of being phosphorylated by a brain protein kinase and this phosphorylation is dramatically stimulated by cyclic AMP. These studies have also demonstrated that ribosomes at all ages can be phosphorylated to the same degree; no differences in phosphate acceptor ability of ribosomes were noted from birth to 1 year of age.

A protein synthesizing system has been developed which permits control over the various components in the protein biosynthetic process. In this optimalized system addition of cyclic AMP and cyclic GMP produced a moderate stimulation of protein synthesis, but only at elevated concentrations. In this same system ribosomes phosphorylated by the cAMP-stimulated protein kinase will be tested for their protein synthetic ability.

Significance to Bio-medical Research and the Program of the Institute: Protein synthesis is a key process involved in the development and maturation of all tissues, including the brain, as well as in the renewal of their intracellular components and in regulation of their biochemical processes in response to physiological, pharmacological, and pathological stresses. A number of syndromes of mental retardation are clearly the result of some disturbance in the process of protein synthesis or its regulation in the brain during its maturation. It is hoped that these studies will shed some light on the normal mechanisms of regulation of protein synthesis in the brain during maturation so that more directed and relevant studies of the biochemical bases of the abnormalities in mental retardation can be undertaken. Furthermore, a good deal of current evidence suggests that cyclic AMP and cyclic GMP might be the intracellular mediators of the putative neurotransmitters, norepinephrine and acetylcholine, respectively. Alterations in the concentrations of both of these transmitters in certain affective disorders and following the administration of psychoactive drugs have been documented. At the present time it is unknown how the neurotransmitters exert their effects on brain function. Effects at the level of protein synthesis could have wide ranging consequences on many aspects of neuronal functioning. The present experiments are designed to determine whether such alterations in protein biosynthesis can in fact be brought about as a consequence of alteration in the concentration of cyclic AMP or cyclic GMP.

Proposed Course of Project: The immediate goal is to determine if cyclic AMP-stimulated phosphorylation of ribosomal proteins alters the ability of the ribosomes to function in protein synthesis, and to determine if such an effect is age dependent. If a stimulatory or inhibitory effect is found, the mechanism of the effect will be explored by determining what control steps in the schema of protein synthesis are affected by ribosomal phosphorylation.

Honors and Awards: None

Publications: None

Serial No. M-CM-MyC-1
1. Laboratory of Cerebral Metabolism
2. Section on Myelin Chemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical studies on myelin and myelin basic protein

Previous Serial Number: Same

Principal Investigator: Russell E. Martenson

Other Investigators: Marian W. Kies and Gladys E. Deibler

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	2.0
Others:	1.5

Project Description:

Objectives: To examine the relationships between chemical structure and biological activities of the myelin basic proteins of several mammalian and submammalian species. The biological activities include the ability to (A) induce delayed hypersensitivity leading to an autoimmune disease, experimental allergic encephalomyelitis (EAE), (B) induce delayed hypersensitivity without necessarily causing EAE, (C) induce the formation of circulating anti-basic protein antibodies without concomitant induction of EAE, (D) serve as a natural acceptor for methyl groups transferred by the enzyme arginine methyltransferase, (E) serve as an acceptor of N-acetylgalactosaminyl groups transferred by an enzyme in brain, N-acetylgalactosaminyltransferase, and (F) provide structural stability in myelin by its interaction with lipids in the membrane.

Methods Employed: (1) Polyacrylamide gel electrophoresis; (2) amino acid analysis of CNS proteins and their derivatives; (3) chromatographic fractionation of proteins and peptides; (4) liquid scintillation techniques for detection of radioactivity, and (5) specific chemical and enzymic cleavage of myelin basic proteins.

Major Findings: (A) Methylation: Myelin basic proteins contain a methylated arginyl residue located at position 107 in the polypeptide chain. A chromatographic method developed by Mrs. Deibler for the direct determination of methylarginines as well as other methylated basic amino acids in total acid hydrolyzates of proteins has enabled us to determine the methylarginine contents of myelin basic proteins of a variety of species, from fish to primate.

Of the two isomers of dimethylarginine, only the symmetrical one has been found in myelin basic proteins from adult animals. In most mammalian, as well as chicken and turtle, basic proteins, there are roughly equal amounts of symmetrical dimethylarginine (SDMA) and monomethylarginine (MMA) which together account for methylation of 50-60% of the protein molecules at this single locus in the polypeptide chain. In the two rodent species which we have examined (rat and guinea pig) the extent of methylation is somewhat less, with most of the methylated arginine present as MMA. Carp myelin basic protein contains no methylarginine and frog myelin basic protein contains only a trace, present as SDMA.

In order to determine if the content of methylarginine in the basic protein changes during myelination, we have analyzed the basic protein isolated from adult and from the newborn miniature pig, the brain of the latter just beginning to undergo myelination. The basic protein of the newborn contains only one-fourth as much SDMA as the adult, and in contrast to all other myelin basic proteins studied so far, that of the newborn contains the unsymmetrical isomer as well.

A second, less time consuming, method for determination of methylarginines has also been developed by Mrs. Deibler which can be used routinely in the standard amino acid analyses of proteins. This procedure is particularly advantageous for the routine analysis of various chemically and enzymically derived fragments of the basic protein since the methylarginines are present at only one position in the polypeptide chain and hence serve as a marker.

Because it has been reported that adult myelin basic proteins contain the unsymmetrical dimethylarginine (UDMA) as well as SDMA, we have examined impure preparations of myelin basic protein and found that they do indeed often contain UDMA but this isomer is always associated, not with the basic protein, but with high molecular weight nonbasic contaminants which can be removed from the myelin basic protein by further purification. An exception, as mentioned earlier, is the presence of UDMA in the protein isolated from newborn miniature pig.

(B) Preparation of Defined Fragments of Myelin Basic Protein Produced by Specific Chemical and Enzymic Cleavages: By specific chemical cleavages with cyanogen bromide and BNPS-skatole and by specific enzymic cleavage with pepsin, it has been possible to obtain the following fragments of the 170-residue basic protein (first and last amino acid residue numbers given): 1-20, 1-116, 21-116, 117-170, 117-167, 90-170, 90-116, 1-44, 45-89. Several of these fragments are presently being utilized by other investigators in our laboratory to define the regions of the basic protein molecule involved in the production of antibody and induction of delayed hypersensitivity. Some of the fragments are also being tested for their ability to induce EAE in the rat and the monkey.

Significance to Biomedical Research and the Program of the Institute: One of the major events occurring during the maturation of the nervous system is the spiral wrapping of modified glial cell membrane around the axons of nerves. This membrane wrapping condenses to form a lamellar insulating structure, myelin, which increases the conduction velocity of nerve impulses. The basic protein, which accounts for 30% of the myelin proteins, appears to have an essential role in the formation and stabilization of the myelin sheath. The protein has a number of unusual characteristics, presently under intensive study in our laboratory, which are undoubtedly related to its structural role, and it also has the unique property of being capable of inducing an autoimmune disease in a variety of animals. It has been the purpose of this laboratory to explain the functions of myelin basic protein in terms of its molecular structure.

Proposed Course of Project: Of particular interest with regard to the structural role of myelin basic protein is the methylation of a single arginyl residue, Arg 107. Brain is relatively rich in an enzyme system which methylates protein arginyl residues. Although this system has been shown by other investigators to methylate Arg 107 of the myelin basic protein, nothing is presently known regarding the specificity of the enzyme which can account for the extent of methylation of Arg 107, the relative proportions of SDMA and MMA, and the absence of UDMA in most species.

Also nothing is presently known concerning the recognition of Arg 107 which determines the enzymic methylation of that residue. The analytical methods which we have developed together with our ability to monitor ^{14}C-labeled amino acids during an amino acid analysis should enable us to investigate the specificity of methylation *in vitro*, both with intact myelin basic protein and with fragments of the protein containing Arg 107; for example, fragments 1-116, 90-170, and 90-116. These studies should enable us ultimately to specify the particular amino acid sequences involved in the recognition of Arg 107 by the methylating enzyme as well as to determine if the unique proportions of the various methylarginines are produced *in vitro* as well as *in vivo*.

The fragment 90-116 also contains a specific threonyl residue which is an acceptor of N-acetylgalactosaminyl groups. Determination of the amino acid sequence recognized by the N-acetylgalactosaminyltransferase enzyme should likewise be possible.

Honors and Awards: None

Publications:

Deibler, G. E., Martenson, R. E., and Kies, M. W.: Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. *Prep. Biochem.* 2: 139-165, 1972.

Martenson, R. E., Deibler, G. E., Kies, M. W., Levine, S., and Alvord, E. C., Jr.: Myelin basic proteins of mammalian and submammalian vertebrates: encephalitogenic activities in guinea pigs and rats. *J. Immunol.* 109: 262-270, 1972.

Morris, J. A., Martenson, R., Deibler, G., and Cagan, R. H.: Characterization of monellin, a protein that tastes sweet. *J. Biol. Chem.* 248: 534-539, 1973.

Deibler, G. E. and Martenson, R. E.: Determination of methylated basic amino acids with the amino acid analyzer. Application to total acid hydrolyzates of myelin basic proteins. *J. Biol. Chem.* 248: 2387-2391, 1973.

Deibler, G. E. and Martenson, R. E.: Chromatographic fractionation of myelin basic protein. Partial characterization and methylarginine contents of the multiple forms. *J. Biol. Chem.* 248: 2392-2396, 1973.

Serial No. M-CM-MyC-2
1. Laboratory of Cerebral Metabolism
2. Section on Myelin Chemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological studies on experimental allergic encephalomyelitis (EAE)

Previous Serial Number: Same

Principal Investigator: Marian W. Kies

Other Investigators: Michael L. Blum, E. C. Alvord, Jr.

Cooperating Units: University of Washington Medical School, Department of Pathology, Seattle, Wash.

Man Years

Total:	2
Professional:	1
Others:	1

Project Description:

Objectives: Study of structural relationships among myelin basic proteins by means of their antigenic reactivity.

Methods Employed: Induction of precipitating antibody in rabbits. Detection of antibody by immunoelectrophoresis and a new micro precipitin reaction developed for rapid survey of large numbers of antisera. Use of a solid immunoadsorbent to prepare pure (monospecific) antibodies.

Major Findings: Our collection of rabbit antisera induced with myelin basic protein has become increasingly useful for studies of structure and function of myelin basic protein. Tests are underway to determine the antigenic specificity of rabbit antisera comparable to those we have described for guinea pig sera in Project No. M-CM-MyC-3. Serum from one of our rabbits immunized with human myelin basic protein has been found by Mrs. Hruby at the University of Washington Medical School to contain antibody which binds specifically residues 117-170 of guinea pig basic protein. Several other sera which did not bind the 117-170 fragment were found to bind residues 1-116 of both bovine and guinea pig proteins.

Preliminary experiments are underway to test the usefulness of our antisera in detecting newly synthesized myelin basic protein in subcellular enzyme systems or in demonstrating synthesis of basic protein in glial cells prior to myelination. Antibody induced by immunization with guinea pig basic protein has been found by Mr. Blum to bind specifically to whole bovine myelin. This has been shown tentatively by the increased protein content of myelin incubated in antisera (presumably γ-globulin). Myelin incubated in normal serum did not bind any serum protein. At least in myelin, those sites in basic protein which bind antibody molecules are different from sites bound to other membrane components.

Specific absorption of anti-basic protein antibody from rabbit antiserum by myelin was also demonstrated by our microprecipitin test. Antiserum which gave a strong positive test with basic protein before incubation with myelin gave a negative test after the myelin had been removed from the serum by centrifugation.

Significance to Biomedical Research and the Program of the Institute: Highly specific precipitating antibody is a powerful tool in the investigation of details of protein structure. This technique will detect minor but biologically significant differences among the myelin basic proteins of various species.

Induction of precipitating antibody is also the most sensitive technique known to biologists for the detection of traces of contaminating proteins in a purified protein preparation.

Proposed Course of Project: The new micro-technique can be used for surveys of human sera. The primary use of the rabbit antisera will be to assist us in further structural studies of myelin basic protein, particularly with respect to cross-reactivity among myelin basic proteins of various species and for the identification of impurities in various preparations of myelin basic protein.

We have been asked to collaborate on a study of *in vitro* synthesis of myelin basic protein which will require a specific antigen-antibody reaction to prove the identity of the newly synthesized proteins. The results described above suggest that such a study is feasible with some of our strong antisera.

Honors and Awards: None

Publications:

Kies, M. W., Martenson, R. E., and Deibler, G. E.: Myelin basic proteins. In Davison, A. N., Morgan, I. G., and Mandel, P. (Eds.): *Structural and Functional Proteins of the Nervous System.* Advances in Experimental Medicine and Biology, Vol. 32, New York, Plenum Press, 1972, pp. 201-214.

Kies, M. W. and Bump, E. A.: A rapid qualitative test for detection of precipitating antibody to myelin basic protein. *Res. Commun. in Chem. Pathol. Pharmacol*. 4: 569-579, 1972.

Behan, P. O., Behan, W. M. H., Feldman, R. G., and Kies, M. W.: Cell-mediated hypersensitivity to neural antigens. *Arch. Neurol.* 27: 145-152, 1972.

Serial No. M-CM-MyC-3
1. Laboratory of Cerebral Metabolism
2. Section on Myelin Chemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on immediate and delayed hypersensitivity in EAE

Previous Serial Number: Same

Principal Investigator: Bernard F. Driscoll (Guest Worker)

Other Investigators: Marian W. Kies and
Allan J. Kramer (Guest Worker)

Cooperating Units: National Multiple Sclerosis Society

Man Years

Total:	3.5
Professional:	1.0
Others:	2.5

Project Description:

Objectives: To study the pathogenesis of EAE at the cellular level and to study the relationship between cellular and humoral responses to myelin basic protein.

Methods Employed: Induction of EAE has been used as a tool to study mechanisms of prevention or treatment of autoimmune disease. Studies of treatment with myelin basic protein have been extended by utilizing studies of the ability of specific peptide fragments to substitute for whole protein in treatment or prevention. The same fragments have been used to localize the sites in the molecule responsible for binding to anti-basic protein antibody.

Major Findings: We reported previously that Strain 13 guinea pigs with EAE had been successfully treated by injections of myelin basic protein in incomplete Freund's adjuvant. The treatment of outbred NIH guinea pigs was not so successful. We recently found that outbred NIH guinea pigs can also be treated successfully if the level of sensitization is somewhat reduced. Their variable response to sensitization made the treatment of EAE technically difficult in the outbred NIH guinea pigs. We have continued studies of the treatment of Strain 13 guinea pigs with EAE and have preliminary observations on the ability of various purified fragments of the guinea pig myelin basic protein to treat. It is apparent that the whole molecule is much more effective than any of the fragments thus far used. These include a basic protein with a substitution at the single tryptophan (therefore, nonencephalitogenic), the small rat myelin basic protein (nonencephalitogenic), and fragments 1-21, 1-44, 45-89, 1-116, 117-170 of the guinea pig basic protein, none of which are encephalitogenic in guinea pigs. While some fragments may have had slight activity in treatment, the presence of the major encephalitogenic site appears to be the most important requisite for treatment. Fragments with sites that have some slight encephalitogenicity (HNB-BP, 1-116, 45-90) treat to lesser degrees and fragments with no known encephalitogenic sites (117-170 and 1-21) fail to treat at all. We have not yet had sufficient quantities of the encephalitogenic tryptophan-containing peptide to test its efficacy in treatment.

In contrast to treatment, prevention of EAE appears to require only an antibody-inducing site in the fragment. The highly encephalitogenic tryptophan-containing nine residue peptide capable of inducing EAE in guinea pigs will not prevent EAE when compared with whole protein on a molar equivalent basis. Other fragments which we have found to have antibody inducing sites in them do prevent EAE and the ability to prevent appears to correlate with their ability to produce antibody.

To round out the picture of immunologic reactivity of myelin basic protein, intensive work on the induction and specificity of its antibody has been performed. Depending on the type and schedule of injections, sera can be prepared which contain antibody specific for a given region of the molecule. Thus far, we have delineated at least three major and one minor antibody-inducing sites in the basic protein molecule. These include major sites between residues 21-89, 117-170, and 90-116 (the most antigenic site) and a minor site in residues 1-21.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the mechanisms involved in the induction, prevention, and cessation of immunologic reactions to myelin basic protein as well as the interrelationships between cellular and humoral immunity should prove to be useful in the study of many neurological diseases, including multiple sclerosis. Even if the precipitating event in these diseases does not involve an autoimmune reaction, they probably are related to an immune reaction to a virus or virus-induced antigen in the cell membrane.

Proposed Course of Project: Further studies on the mechanism of prevention and treatment and the relation between the two are planned.

New and smaller fragments of the myelin basic protein are at present being prepared and purified. With these, it will be possible to delineate more closely the sites of the molecule responsible for treatment, prevention, and/or antibody induction. We believe that treatment and induction of disease are somehow related while antibody specificity and prevention are associated. The regions responsible for the former (treatment and induction) are different from the regions responsible for the latter. The current trend in the literature on EAE is to confuse these phenomena.

Honors and Awards: None

Publications:

Kies, M. W.: The biological activity of myelin basic proteins. In Wolfgram, F., Ellison, G. W., Stevens, J. G., and Andrews, J. M. (eds.): Multiple Sclerosis. Immunology, Virology, and Ultrastructure. New York, Academic Press, 1972, pp. 429-448.

Kies, M. W., Driscoll, B. F., Seil, F. J., and Alvord, E. C., Jr.: Myelination inhibition factor: dissociation from induction of experimental allergic encephalomyelitis. Science 179: 689-690, 1973.

Serial No. M-CM-MeC-1
1. Laboratory of Cerebral Metabolism
2. Section on Membrane Chemistry
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of the modulation of central nervous system metabolism and function through alterations in membrane permeability and transport

Previous Serial Number: Same

Principal Investigator: William C. Torch, M.S., M.D.

Other Investigators: Harry R. Steinberg, M.D., Ana Maria Lennon, Ph.D., James D. Brown, Carolyn S. Cottingham, and Louis Sokoloff, M.D.

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	2.0
Other:	1.5

Project Description:

Objectives: Neurotransmitters might exert their effects in the central nervous system (CNS) by altering the basic chemical structure of CNS membranes. This might occur through transient and/or permanent alterations in the lipid and protein composition via metabolic mechanisms as well as through intramembranar lipid-protein phase changes, either of which might affect ion permeability and electrical excitability. Previous workers in this laboratory have already demonstrated that the neurotransmitter, acetylcholine (ACh), can affect phospholipid synthesis and degradation in CNS membranes (synaptosomes). The molecular mechanism by which ACh causes these effects has been the intense objective of study of this laboratory. Specifically, acetylcholine has been demonstrated to increase the rate of incorporation of radiophosphate into phospholipids, particularly phosphatidylinositol (PI) and phosphatidic acid (PA). In addition ACh has been shown to enhance the hydrolysis of PI and its derivatives diphosphophosphatidylinositol (DPI) and triphosphophosphatidylinositol (TPI) into their inositol phosphate breakdown products. Calcium has been shown to affect the rate at which ACh-mediated phospholipid synthesis and degradation occurs. The mechanism by which ACh alters phospholipid synthesis has still further to be elucidated.

It has been noted in the literature that norepinephrine, as well as other putative neurotransmitters, has the same effects as ACh on phospholipid metabolism, that is, the "phospholipid effect" is not specific to ACh. Moreover it has been noted over the past few years that some cyclic nucleotides might be specific intermediate mediators between intramembranar metabolic reactions. Cholinergic mechanisms may be specifically mediated through the cyclic GMP guanylylcyclase enzyme system and adrenergic mechanisms through the cyclic AMP adenylylcyclase enzyme system. The question that logically follows, and to which this project is addressed, is whether acetylcholine and cholinergic drugs affect cholinergic mediated phospholipid synthesis through the cyclic GMP system, and whether norepinephrine and other adrenergic drugs express their effects on phospholipid metabolism through the cyclic AMP system.

Methods Employed: The project is to be approached in 3 stages, the first two of which are already in progress.

1. To determine if cyclic GMP and cyclic AMP and their derivatives can in themselves affect phospholipid metabolism in synaptosomes. Also to determine whether cyclic GMP and cyclic AMP can affect the cholinergic and adrenergic mediated phospholipid response induced by acetylcholine and norepinephrine, respectively.

2. To determine whether ACh and norepinephrine and other putative neurotransmitters (epinephrine, serotonin, histamine, dopamine, GABA) do indeed affect levels of the cyclic nucleotides in synaptosomes. In order to do this methods for the separation, purification and measurement of cyclic AMP and cyclic GMP will be established.

3. To determine whether the phospholipid response mediated by ACh and the other neurotransmitters is limited to specific areas in the brain that are specialized in specific cyclic nucleotide synthesizing ability.

In Stage 1, synaptosomes isolated from guinea pig cortex by differential centrifugation are incubated for varying time periods in the appropriate radiophosphate-(^{33}P) media containing acetylcholine, cyclic AMP and cyclic GMP in various concentrations. By established lipid extraction techniques the various phospholipids are isolated and purified by thin-layer chromatography and then measured for radioisotope incorporation. A measure of how much effect each of the aforementioned agents has in phospholipid metabolism is obtained.

In Stage 2 synaptosomes isolated from guinea pig cortex will be incubated with each of the putative neurotransmitters. Cyclic nucleotides will be separated and purified by column chromatography. Cyclic AMP will be assayed using a radioisotope displacement technique employing a purified preparation of cyclic AMP dependent-protein kinase. Cyclic GMP will be assayed by measuring the phosphorylation of histone mediated by a purified preparation of cyclic GMP dependent-protein kinase.

In Stage 3 various areas of brain will be assayed for the effects of ACh and other neurotransmitters on cyclic nucleotide production (as in Methods, Stage 2). Also the effect of cyclic nucleotides on phospholipid metabolism in synaptosomes isolated from these brain regions will be measured (as in Methods, Stage 1).

Major Findings: Our working hypothesis is that ACh exerts its phospholipid effects at the membrane surface of synaptosomes by stimulating the production of cyclic GMP by direct activation of intramembranar guanylylcyclase. Cyclic GMP might then interact at some level of phosphatidylinositol metabolism causing a markedly increased turnover of phosphatidylinositol and its precursor, phosphatidic acid. Likewise norepinephrine and histamine might enhance cyclic AMP production through similar mechanisms involving adenylylcyclases. Cyclic AMP might then have similar phospholipid effects.

Evidence supporting this hypothesis was obtained in preliminary experiments in which synaptosomes isolated from guinea pig cortex were labeled with radiophosphate (^{33}P). Incorporation into PA and PI was stimulated by ACh and cyclic AMP. The effects of cyclic GMP were dose dependent. High concentrations were inhibitory and low concentrations stimulatory. Derivatives of cyclic AMP and cyclic GMP likewise have been found to affect phospholipid metabolism. The ability of the cortex to respond to both cyclic AMP and cyclic GMP in a similar non-specific manner may be a reflection of the presence of both cholinergic and adrenergic receptors in this area of the brain.

Significance to Bio-medical Research and the Program of the Institute: The mechanism of action of neurotransmitters in the CNS and other target organs is presently unknown. In addition to the theoretical interest of how transmitters affect electrical activity in the nervous system, knowledge of the mechanism of hormone action would shed light on the physiological and pathological mechanisms of health and disease in the nervous system. Recently it has become apparent that altered levels of cyclic nucleotides are observed in altered psychologic states, and that psychotropic drugs can alter levels of cyclic nucleotides. Knowledge of the mechanism would help to elucidate how pharmacologically active drugs work, and would provide a rational basis for the development of new drugs to be used in treating disease.

Proposed Course of Project: Further studies on the mechanism of action of neurotransmitters and their biochemical intermediates on phosphoinositide and phospholipid metabolism in the CNS are to be continued as described in Methods.

Honors and Awards: None

Publications:

Lennon, A. M., and Steinberg, H. R.: Evidence for involvement of Ca^{2+} in the incorporation of $^{32}P_i$ into the phosphatidylinositol of rat diaphragm. J. Neurochem.20: 337-345, 1973.

Serial No. M - LGCB 16B
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSHMA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Amino Acid tRNA Synthetases. Their Role In Protein Synthesis, and Interaction With tRNA

Previous Serial Number: M-LGCB 16 and 16A

Principal Investigator: Giulio L. Cantoni

Other Investigators: Elizabeth A. Boeker

Cooperating Units: Dept. of Biochemistry, University of Washington

Man Years:

Total: 3/4
Professional: 1/4
Other: 1/2

Project Description:

Objectives: Aminoacyl tRNA synthetases catalyze the first reaction of protein synthesis:

$$\text{Amino acid} + \text{ATP} + \text{Synthetase} \rightleftarrows \text{Aminoacyl-tRNA} + \text{AMP} + \text{Synthetase}$$

$$\text{Amino acid} + \text{ATP} + \text{tRNA} \overset{\text{Synthetase}}{\rightleftarrows} \text{Aminoacyl-tRNA} + \text{AMP} + \text{P}{\sim}\text{P}$$

The specificity of the translation of genetic information from DNA into linear polypeptide chains depends critically on the recognition by aminoacyl tRNA synthetases of their specific amino acids and cognate tRNA molecules. Although each tRNA has a unique base sequence, the secondary and tertiary structures of these nucleic acids are all rather similar, and the mechanism by which a specific synthetase recognizes the correct tRNA is not known. The specificity of this recognition cannot reside in the anti-codon unique to each tRNA since, in several cases, a single synthetase recognizes multiple tRNA's, all coding for the same amino acid but having different anti-codons. The interaction between tRNA and aminoacyl tRNA synthetases thus provides an ideal system for investigation of the mechanism by which proteins recognize nucleic acids, a mechanism central to a variety of problems of great biological importance.

The aminoacyl tRNA synthetases also represent a family of enzymes which catalyze formally similar reactions with different substrate specificities. A class of enzymes such as this might be expected to show rather similar structural parameters, but simple structural analysis has already shown that this is not the case. Based on the available data for the molecular weight and subunit structure of less than half of the 20 synthetases, at least three classes are distinguishable: monomers with a molecular weight of approximately 100,000, dimers with a total molecular weight also approximating 100,000, and tetramers with a total molecular weight around 180,000. The data currently available are not sufficient to interpret these differences meaningfully, and a great deal of additional information will be required to achieve an adequate understanding.

Methods Employed: Amino acid activation can be measured either by the incorporation of ^{32}P~P into ATP (exchange reaction) or the incorporation of ^{14}C-serine into tRNA (acylation reaction). Classical methods of enzymology and protein chemistry are used in the purification and structural characterization of the synthetase. In a particularly useful feature of this system, both the aminoacyl-AMP-synthetase complex, formed in the absence of tRNA, and the synthetase-tRNA complex, formed in the absence of ATP and serine, can be isolated by gel filtration or trapped on nitrocellulose filters, allowing independent evaluation of the characteristic of each interaction. The information thus obtained using substrate levels of enzyme complements that obtained from kinetic analyses using catalytic quantities of the synthetase.

Major Findings: The serine tRNA synthetase of *E. coli* B has been purified to homogeneity; the procedure is adequate for the isolation of sufficient enzyme to carry out structural studies. The enzyme is essentially homogeneous by the criteria of acrylamide gel electrophoresis and isoelectric focusing.

To investigate the enzyme-substrate complexes formed by the seryl tRNA synthetase, we have employed equilibrium dialysis and have modified the gel filtration procedure so that it is carried out under steady-state conditions, allowing quantitative treatment of the data. In the absence of serine and tRNA, an enzyme-ATP complex ($K_a = 4 \times 10^4\ M^{-1}$) is formed; the corresponding enzyme-serine complex has not been detected ($K_a < 5 \times 10^{-3}\ M^{-1}$). In the absence of tRNA, an enzyme-seryl-AMP complex is formed; the stoichiometry of both complexes is 1 mole of substrate(s) per 53,000 g.

An extensive ultracentrifuge investigation of the free enzyme and both complexes has revealed no substantial differences in behavior among the three species. All have a molecular weight of ca. 100,000, a sedimentation coefficient, $s^o_{20,w}$, of 5.4 S, and a tendency to dissociate at low protein concentrations. Gel electrophoresis in sodium dodecyl sulfate and isoelectric focusing in urea indicate that the native enzyme is composed of two apparently identical subunits with a molecular weight of 53,000 ± 3000. We conclude that the native enzyme is a dimer having two identical subunits and two independent active sites.

At least 8 kinetically distinguishable mechanisms can be written for the exchange of PP_i^{32} into ATP; rate equations expressing the explicit dependence of the initial exchange rates on substrate concentrations have been developed and tested. The results indicate that the exchange reaction proceeds with ordered addition of substrates (ATP first) and two detectable ternary complexes, enzyme-serine-ATP and enzyme-seryl-AMP. Additional kinetic measurements in the presence of tRNA show that the acylation reaction proceeds by a similar mechanism.

Scientific Significance: Interaction between nucleic acids and proteins is an important biological phenomenon. The interaction of an amino acid tRNA synthetase with the corresponding tRNA in a highly purified system may afford a useful model for such interactions in general. In addition, this study should provide important information about the complex two-step reaction catalyzed by the amino acid tRNA synthetases.

Proposed Course of Research: These studies will be continued as they are part of the Laboratory's long-range program on the nucleic acid protein recognition and interactions.

Honors and Awards: None

Publications:

Boeker, E. A., Hays, A. H., and Cantoni, G. L.: Seryl tRNA synthetase of *E. coli* B: Purification, subunit structure, and behavior in the acylation reaction. *Biochemistry*, in press.

Boeker, E. A., and Cantoni, G. L.: Seryl tRNA synthetase of *E. coli* B: Enzyme substrate interactions. *Biochemistry*, in press.

Serial No. M - LGCB 18
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the Structure and Function of Thetin-Homocysteine Methylpherase and Lactose Synthetase

Previous Serial Number: Same

Principal Investigator: Werner A. Klee

Other Investigators: K. Subramonia Iyer and Claude B. Klee

Cooperating Units: Laboratory of Biochemical Pharmacology, NIAMDD

Man Years:

Total:	2
Professional:	1-1/2
Other:	1/2

Project Description:

Objectives: Macromolecular complexes on many levels are a central principle of biological organization. This project is aimed at learning how the properties of such complexes can differ from those of the isolated components in two enzyme systesm. One enzyme, thetin-homocysteine methylpherase, exhibits a number of interactions of like protein species; the other, lactose synthetase, is an example of an enzyme in which two very different protein species interact to form the whole.

Methods Employed: We are using the standard methods of protein chemistry including chromatography, electrophoresis, preparative and analytical ultracentrifugation, spectrophotometry and spectropolarimetry. In addition, many of the standard analytical techniques of enzymology are being employed.

Major Findings: Lactose synthetase consists of two protein components both of which are found in lactating mammary glands and in milk. The two proteins are the A-protein and α-lactalbumin. We have isolated the A-protein from milk and shown it to be a mixture of closely related glycoproteins.

The A-protein alone can carry out galactose transfer reactions from UDP-galactose to a variety of glycoproteins, glycolipids and to some simple sugars such as N-acetlyglucosamine. The effect of α-lactalbumin on the system is to produce a dramatic increase in the affinity for sugar acceptors be they glucose or N-acetylglucosamine. This simple picture is complicated by the existence of a substrate inhibition seen at high concentrations of acceptor sugars. α-lactalbumin enhances this effect as well and so is seen to stimulate or inhibit glycosyl bond formation depending on the acceptor sugar concentration.

These kinetic observations made it appear highly likely that α-lactalbumin functions by combining with the A-protein to form a multisubunit complex. We have found that such complexes are indeed formed but only in the presence of one of the substrates of the enzyme, either UDP-galactose or N-acetylglucosamine. Complex formation was demonstrated by a number of methods including a newly developed modification of band sedimentation in the analytical ultracentrifuge. The complex contains one molecule of A-protein and one molecule of α-lactalbumin and is quite easily dissociated. Although α-lactalbumin is the only protein known to function in this system, it has been found that the only modifications to its structure which will prevent interaction with the A-protein are major ones such as disulfide bond reduction or extensive proteolysis. Modification of amino acid residues such as tyrosine, methionine, histidine and at least some aspartic and glutamic acids results in only a minor decrease in activity. Thus, the interaction of the two proteins may involve the mutual recognition of large areas of their surfaces. Studies of the forces involved in stabilizing the complex show that ionic interactions may play a major role, and that hydrophobic bonding is probably not an important contributor to the binding energy.

During the course of a study of the guanidination of α-lactalbumin by o-methylisourea, a number of important properties of the protein have been uncovered. We can now say that all 12 lysine residues of the protein can be converted to homoarginine without sensibly changing its overall structure or function. Thus, lysine residues, as such, are not an essential part of the interaction of α-lactalbumin and the A-protein. A side reaction during guanidination is disulfide interchange leading to polymer formation and loss of function. α-lactalbumin is unique in this respect and it was therefore plain that its disulfide bonds exhibit an unusual degree of reactivity. Measurement of the rate of reduction of the four disulfide bridges of α-lactalbumin, with the aid of a direct spectrophotometric assay developed by us for this purpose has shown that the disulfides of this protein are indeed extraordinarly reactive. In particular, one disulfide bridge, linking cysteinyl residues 6 and 119 at the amino acid carboxyl termini of the molecule is highly reactive even at 0^{o}. The other three disulfides are reactive at room temperature and above but not at low temperatures. These results bring into question the similarity of structure of α-lactalbumin and lysozyme which has been postulated on the grounds of amino acid sequence homology.

An activity similar to or identical with that of the A-protein found in milk has been reported to be present in particulate fractions of many tissues. We have therefore initiated an examination of the properties of this enzyme in a number of tissues but have concentrated our attention on brain. In the rat brain we have found appreciable amounts of A-protein which are associated primarily with the synaptosomal fractions, as isolated by differential centrifugation techniques. The enzyme activity associated with these particles is not sensitive to modification by α-lactalbumin unless it is first converted to a soluble protein with detergent treatment. This solubilized enzyme, from either rat or bovine brain is kinetically indistinguishable from the A-protein in bovine milk. It may readily be purified by affinity chromatography on columns of α-lactalbumin bound to sepharose.

Scientific Significance: The lactose synthetase system represents a novel type of evolutionary adaptation in which an enzyme which is normally used for glycoprotein and glycolipid biosynthesis (the A-protein) is modified to accomplish a new function, lactose biosynthesis, by complexing with another, non-catalytic protein, α-lactalbumin. Thus, the system is a clear example of how protein function can be modified in a dramatic way by incorporation into more complex structures. Furthermore, the A-protein is known to be associated with membrane systems such as synaptosomes and liver cell membranes and may have an important role in membrane synthesis or function.

Honors and Awards: None

Publications:

Iyer, K. S., and Klee, W. A.: Direct spectrophotometric measurement of the rate of reduction of disulfide bonds. The reactivity of the disulfide bonds of bovine α-lactalbumin. J. Biol. Chem. 248: 707-710, 1973.

Serial No. M - LGCB 35
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Protein Conformation and Limited Proteolysis

Previous Serial Number: Same

Principal Investigator: Werner A. Klee

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives: Although it is now a well established principle that the linear sequence of the amino acids of a polypeptide chain determines the nature of the characteristic structure (conformation) of proteins, the process whereby this transformation of two dimensional information to a three dimensional structure occurs is not well understood. The ultimate goal of this project is to supply experimental data which may lead to some insight into this problem. A closely related question which we would like to help answer is: What is the nature of the conformational changes which take place in protein systems and what is the role of such structural flexibility in the functioning of macromolecular systems?

Methods Employed: One way of finding out something of the nature of the conformational determinants of proteins is to study the properties of smaller fragments of the molecules. We have prepared a number of peptides of overlapping sequence from three proteins by a combination of proteolytic and chemical cleavage, and are studying the conformational properties of these materials by physical methods which include spectropolarimetry, ultraviolet and nuclear magnetic spectroscopy, ultracentrifugation and titrimetry. These studies are performed over a range of solvent conditions and temperatures and provide information on the conformation of these peptides in solution which can then be compared with the known structures of these materials when they are part of the intact protein in the crystalline state. We also study the nature of the conformational changes in which intact proteins participate on variation of the state of their environment or as a result of interactions with other kinds of molecules. This work uses the same kinds of physical methods described above in conjunction with some more subtle probes of structure such as limited proteolytic cleavange of exposed (and unstructured) regions.

Major Findings: We have studied in detail the conformational properties of a number of peptide fragments of ribonuclease, of insulin and of α-lactalbumin. The peptides chosen for study to date are all fragments containing the amino terminus of the protein and vary simply in the length of the rest of the polypeptide chain which remains attached. In this way a comparison of the properties of closely related peptides gives information about the structure determining role of successive regions of the polypeptide sequence. The results of this work to date are consistent with the hypothesis that those portions of the polypeptide chain of the proteins which are found to be in a helical region of the intact protein participate in a helix-like structure in solution as well. In isolation, these helices have rather different stability properties from those of the protein and may be more appropriately considered to be "nascent" helices rather than fully formed ones. Peptide segments which do not participate in a repeating structure in the intact protein appear to be essentially disordered in solution. An analysis of the published data from other laboratories with peptides derived from other proteins indicates that the phenomenon of "nascent helix" formation by fragments of helical regions of intact proteins may be a general one.

Our work with ribonuclease fragments had shown that the nascent helix near the amino-terminus of the molecule is stable only at low temperatures when fragments of short length are studied. The behavior of the nascent helix of the insulin B chain, (which corresponds to the amino terminus of proinsulin) is interesting in that it is stable only at high temperatures (37^{o}) and becomes disordered as the temperature is lowered. It thus appears that there may be more than one kind of nascent helix which is formed. It may be that the structure in the case of the insulin fragment is stabilized largely by hydrophobic interactions whereas that of the ribonuclease fragment is stabilized primarily by other kinds of forces.

The three dimensional structure of α-lactalbumin (see Project M-LGCB 18) has so far eluded solution by x-ray crystallographic methods. The amino acid sequence of this protein is, however, known to be very closely related to that of the well characterized protein antibiotic, lysozyme and it has been proposed that the conformation of the two proteins should therefore be similar. Studies of the physicochemical properties of the two proteins in this and other laboratories support this hypothesis. We have now succeeded in preparing mixed crystals of the two proteins which are stabilized primarily by salt linkages and appear to be composed of α-lactalbumin and lysozyme in the molar ratio of 1:2. It is possible that the availability of such a mixed crystal system will facilitate the x-ray analysis of α-lactalbumin structure.

Scientific Significance: These studies are providing a stock of techniques which may be generally useful in studying the nature of the determinants of protein structure. On the basis of our results it would appear that short range interactions, among near neighbor amino acid residues, control the nature of the protein structure to an important degree. The specific types of short range interactions involved are yet to be determined but it would appear that there may be at least two distinct ways of specifying helical structures.

Proposed Course of Research: We intend to continue these studies along the general lines discussed above.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 56
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Determination of the Effect of Small Viruses and Their Nucleic Acids On the Biochemistry of Living Organisms

Previous Serial Number: Same

Principal Investigator: Carl R. Merril

Other Investigators: T. Friedman, K. Krell, M. Gottesman, J. Petricciani, M. Geier, I. Prissovsky, R. Yarkin

Cooperating Units: Laboratory of Molecular Biology, NCI; Pathology Laboratory, Bureau of Biologics, FDA

Man Years:

Total:	6
Professional:	3
Other:	3

Project Description:

Objectives: An attempt to determine the genetic information contained within the nucleic acids of small "viruses" and their effect on various organisms.

Methods Employed: Biochemical techniques for purification and analysis of small viruses and nucleic acids, ultraviolet spectroscopy, density gradient ultracentrifugation, bacteriophage genetics, radioactive tracer techniques, mammalian tissue culture methods and fruit fly developmental genetics.

Major Findings: 1. By using a bacteriophage carrying bacterial genes (the galactose operon), it was possible to infect human cells, fibroblasts, cell line CCl-72, with a defect in galactose metabolism, and to demonstrate a change in their galactose metabolism. This work has been extended to three new galactosemic human cell lines: GM52, GM53 and GM54. In a series of 28 independent experiments, cells were infected with λpgal ($K^+T^+E^+$); 68% yielded transferase activity. The activity obtained ranged from 0.2 to 35% of the activity in normal human cells. The average restoration was 1% of normal. Studies on the transferase assay system have increased its reliability and sensitivity by a factor of 10. A major effort is now being made to find differences between the bacterial enzyme and the human enzyme so that it can be further shown that the enzyme which appears after infection in the galactosemic human cells is a bacterial enzyme.

2. In an effort to follow the expression of prokaryotic genes through a number of cell generations, it is important to work with cells capable of cloning. Unfortunately, galactosemic fibroblasts do not fulfill this criteria. Chinese hamster ovary cells and mouse L cells can be grown and cloned in chemically defined media. By omission of biotin the cells should not be capable of survival. It is hoped that such cells can be rescued by infection with a biotin transducing bacterial virus λpbio. By cloning rescued cells the permanence of the change can be studied.

3. To study the effect of specific prokaryotic genes in eukaryotic whole organisms, work is continuing on the fruit fly.

4. To study distribution, circulation and destruction of bacterial viruses, these viruses were injected into germ free mammals. This study has yielded general information on virus host interactions. Bacterial viruses are treated in the mouse in a manner equivalent to mammalian virus, or for that matter to any colloidal particle of ~100 Å diameter. Such particles are rapidly destroyed by the R.E. system in the liver. Within two hours after injection, the titer of virus in the blood drops by a factor of 100. This viral destruction can be blocked to some extent by giving a large dose of inert colloidal particals a the same time as the virus.

Scientific Significance: The initial observation, that prokaryotic genes can function in eukaryotic systems, has been confirmed using bacteriophage in plant cells (Doy, Gresshoff and Rolfe, PNAS 70: 723-726, 1973; Carlson, PNAS 70: 598-602, 1973). In these studies, the authors used bacterial viruses which were similar to those employed in our studies, illustrating that plant cells can be altered by such viruses. This is especially significant because if one were able to construct a bacterial virus carrying the nitrogen fixation genes into plant cells, it would become possible to transform atmospheric nitrogen into organic nitrogenous products such as amino acids and proteins. The observation that bacterial viruses can produce proteins in mammalian cells also raises the possibility that these viruses are directly or indirectly involved in human disease. It has already been well established that bacteriophage can indirectly cause human suffering by formation of toxins in bacterial hosts. With the demonstration of direct bacterial virus gene function in eukaryotes, it may be that some bacterial viruses act directly in the mammalian host cells. Mankind has lived in symbiosis with the bacterial *E. coli* in his large intestine for some time. It would be surprising in view of this relationship if there were not some viruses which could not live in both hosts. It may also be that some of these viruses are directly responsible for human disease.

Proposed Course of Research: To continue the study of these viruses and their interaction with other life forms, with emphasis on molecular level events.

Honors and Awards: None

Publications:

Merril, C. R., Friedman, T. B., Attallah, A., Geier, M. R., Krell, K., and Yarkin, R.: Isolation of bacteriophages from commercial sera. *In Vitro* 8: 91-93, 1972.

Binder, M. C., Petricciani, J. C., Merril, C. R., and Geier, M. R.: Aspects of galactose metabolism in normal and galactosemic cell cultures. *Med. Ann. D.C.* 41: 228-230, 1972.

Friedman, T. B., and Merril, C. R.: A microradiochemical assay for urate oxidase. *Anal. Biochem.*, in press.

Serial No. M - LGCB 58
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ribonuclease Specific for RNA-DNA Hybrids

Previous Serial Number: Same

Principal Investigator: Giulio L. Cantoni

Other Investigators: Roy C. Haberkern

Cooperating Units: Children's Hospital Medical Center, Boston

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives: An enzymatic activity from calf thymus extracts has been reported to specifically hydrolyze the RNA portion of RNA-DNA hybrids, with no activity on RNA in other forms. This activity (ribonuclease H) has been purified and further studied.

Methods and Findings: Protein purification and nucleic acid isolation and characterization have been by standard methods. The enzyme has been purified to >80% purity. The enzyme activity has been shown to be a property of a single protein, MW ~ 70-75,000, with no subunits. Substrate specificity has been confirmed using both biologic (viral) and synthetic nucleic acid substrates. The enzyme is located in the cell cytoplasm.

Similar enzymatic activity has been reported in adrenal tissue, mouse tumor cells adapted to culture, and RNA tumor viruses.

Scientific Significance: That an enzyme of such specificity is present in a variety of tissues suggests that its substrate, RNA-DNA hybrid molecules, exist and have a function in cells.

Current notions regarding information transfer in cells do not make clear what function such hybrids, and their hydrolysis by this nuclease, might serve.

RNA reverse transcriptase, the enzyme responsible for the replication of oncogenic RNA viruses has been shown to be associated with ribonuclease H. The study of ribonuclease H from oncogenic RNA viruses will prove important in their biology. Ribonuclease H from calf thymus is extranuclear. Thus, its role can not be related to DNA transcriptase (or RNA reverse transcriptase) since these are nuclear events. RNA-DNA hybrids are not known to occur in the cytoplasm and the function of ribonuclease H in eukaryotic cells is entirely unknown. It will be very interesting to explore the occurrence of ribonuclease H in tissue or the calf thymus and to determine its subcellular distribution. Inhibitor studies with a series of rifamicin derivatives have brought out important differences between the pattern of inhibition of ribonuclease H from eukaryote cells and from oncogenic viruses.

Proposed Course of Research: Further studies of the enzyme and its role are planned. A hypothesis linking ribonuclease H and the post transcriptional control of messenger RNA is being elaborated and will in due course be subject to experimental scrutiny.

Honors and Awards: None

Publications:

Haberkern, R. C., and Cantoni, G. L.: Studies on a calf thymus ribonuclease specific for RNA-DNA hybrids. Biochemistry, in press.

Serial No. M - LGCB 59
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Spermidine Synthesis in Rat Brain

Previous Serial Number: Same

Principal Investigator: Giulio L. Cantoni

Other Investigators: Gregory L. Schmidt

Cooperating Units: None

Man Years:

Total:	1-1/4
Professional:	3/4
Other:	1/2

Project Description:

Objectives: The polyamine spermidine has been implicated as a control factor in several rapid growth processes. This project is designed to determine whether spermidine is also involved in the control of rat brain development.

Methods Employed: Two of the three enzymes involved in the synthesis of spermidine from ornithine and S-adenosylmethionine (AMe), ornithine decarboxylase and AMe decarboxylase, have been isolated from rat brain and characterized to an extent sufficient to allow determination of optimal assay parameters and assays applicable to small amounts of brain tissue have been developed. The activities of both enzymes are measured by determination of $^{14}CO_2$ released from the substrates. These enzymes are measured in rat brain homogenates at 2-3 day intervals following birth and related to known synthetic and anatomical correlates of brain development.

Major Findings: Both ornithine decarboxylase and AMe decarboxylase have pH optima and kinetic parameters similar to those reported in other rat tissues. AMe decarboxylase is a soluble enzyme which requires putrescine for activity but apparently does not require pyridoxal phosphate. Ornithine decarboxylase is a nuclear enzyme for which pyridoxal phosphate is the probable cofactor. Preliminary results indicate that the specific activities of ornithine decarboxylase and AMe decarboxylase increase to plateau levels within several days after birth of the rat then, at an age of approximately two weeks, increase to levels 4-5 fold higher than the initial plateau. These two phases approximately coincide with the time of maximal rates of DNA, RNA, and protein synthesis and the time of maximal synapse formation and myelination, respectively.

Scientific Significance: Polyamines are ubiquitely distributed in bacterial cells, plants and mammalian tissues. The role of polyamines has been widely investigated and several functions have been attributed to them particularly in connection with RNA biosynthesis or DNA transcription. There are important differences between the enzymatic mechanism involved in polyamine biosynthesis in bacteria and in eukaryotes, but the significance of these differences is not yet understood.

Proposed Course of Research: The studies on rat brain S-adenosylmethionine decarboxylase and putrescine propylamine-pherase will continue. The enzyme is being further purified. One of the major uncertainties in the biosynthesis of spermidine in higher animals is the nature of the cofactors involved in the reactions. It is expected that this question will be answered by enzyme purification. A study of the effect of S-adenosylmethionine and thiomethyladenosine analogues, synthesized by Dr. Borchardt, on the S-adenosylmethionine decarboxylation and spermidine synthesis is also planned.

Honors and Awards: None

Publications:

Schmidt, G. L., and Cantoni, G. L.: Ornithine decarboxylase and adenosylmethionine decarboxylase in rat brain: Properties, subcellular distribution and neonatal development. J. Neurochem., in press.

Serial No. M - LGCB 60
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Proteins
3. Bethesda Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1 1972 through June 30 1973

Project Title: Studies on the Biochemical Basis of Narcotic Drug Action

Previous Serial Number: None

Principal Investigator: Werner A. Klee

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives: To study the interaction of morphine like compounds with receptor sites in the nervous system and the significance of such interactions to the function of the nervous system at the biochemical level.

Methods Employed: Subcellular fractionation of brain tissue a number of different binding techniques and measurements of neurotransmitter uptake and release by subcellular fractions.

Major Findings: This project was initiated only in the past few months and our findings are of the most preliminary nature. They are encouraging enough to indicate that we will be able to measure the specific binding of morphine like compounds to their putative receptors and that we may be able to correlate such binding with neurotransmitter transport.

Scientific Significance: This work is of too preliminary a nature to be assessed at this time.

Proposed Course of Research: We intend to continue our studies as outlined above.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 61
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Muscle Differentiation in Tissue Culture

Previous Serial Number: None

Principal Investigator: Giulio L. Cantoni

Other Investigators: Sigfrido Scarpa and B. William Uhlendorf

Cooperating Units: None

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: Embryonic skeletal muscle culture system *in vitro* has been recognized as a potential model of cellular differentiation. The principal differentiative event is the fusion of mononucleated myoblasts into multinucleated myofibers, that then go on to form fully differentiated striated myotubules. The mechanism of myoblast fusion and the factors controlling it are not adequately understood.

Methods Employed: Embryonic muscle is cultured *in vitro* and the degree of differentiation is evaluated by a combination of cytological and biochemical tests. The influence of various components of the culture medium on the course of differentiation is evaluated.

Major Findings and Scientific Significance: Our efforts have been directed toward the elaboration of a biochemical "differentiation index." We are planning to take advantage of the discovery that DNA synthesis ceases after myoblast fusion, to determine the degree of differentiation (= myoblast fusion). We are engaged in the process of developing sensitive micro techniques for the determination of total DNA and of the rate of DNA synthesis in tissue cultures. Also, we are examining the effects of subtle changes in cultural conditions on the course of differentiation.

Proposed Course of Research: We are in the early phases of this project and there are many options to us for future studies. Our course will be determined by the outcome of the exploratory experiments under way.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 62
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: S-Adenosylmethionine Analogues As Tools For Investigation of Biological Transmethylation and Transalkylation Reactions

Previous Serial Number: None

Principal Investigator: Giulio L. Cantoni

Other Investigators: Ronald T. Borchardt

Cooperating Units: University of Kansas, Lawrence, Kansas

Man Years:

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Background and Objectives: Since analogs and derivatives of metabolic intermediates have contributed significantly to the understanding of biochemical reaction mechanisms, the possibilities of modifying the structure of the biological methyl donor, S-adenosylmethionine (AMe), could provide a means of obtaining information concerning the structural requirements at the active site and aid in determining the mechanism of methyl transfer of various methyltransferases.

Since the discovery of AMe in 1952, a great variety of enzymatic transmethylation reactions have been demonstrated *in vitro* by use of this compound. It has been shown that the methyl group of AMe can be transferred to the nitrogen of primary, secondary or tertiary amines or of heterocyclic compounds, the sulfur of mercaptans or thio-ethers, selenium compounds, the oxygen of phenols, carboxylate structures or water, to carbon atoms of a number of compounds and to the cobalt atom of certain corrinoids. From the physiological standpoint, the importance of AMe in the biosynthesis and/or metabolism of biogenic amines, methionine, terpenes, branched fatty acids, sterols and quinones, methylated RNA, methylated DNA, methylated pectin and methylated proteins have been well documented. Of recent interest has been the specific modifications of the properties of the biopolymers consequent to methylation and its importance in the regulation of a variety of biological processes.

For most transmethylation reactions, there is unequivocal evidence that AMe is capable of functioning as the methyl donor.

Substitution of other purine and pyrimidine bases for the adenine moiety of AMe would be of considerable interest to determine if nature has selected only the adenosine sulfonium system or if other nucleoside sulfoniums exist in nature and function in transmethylations. Enzymatic synthesis of this type of analog has presented problems because of the high substrate specificity which the enzyme ATP-L-methionine-S-adenosyltransferase exhibits.

Since synthetic modifications of AMe have revolved predominantly around the amino-acid moiety, further modification of the base would be of considerable interest. The only other work reported on modification of the base portion of AMe has been the preparation of the corresponding inosyl derivatives.

Considering the importance of AMe in biological transmethylations, it would be of interest to determine if other nucleoside sulfonium systems exist in nature and function as methyl donors. Of particular interest would be derivatives of the bases of uracil, thymine, cytosine and guanine. These would be the corresponding S-uridylmethionine, S-thymidylmethionine, S-cytidylmethionine and S-guanosylmethionine. These nucleoside sulfoniums could be utilized to further elucidate the structural requirements at the active site and aid in determining the mechanism of methyl transfer of various methyltransferases, as well as determine if these substances occur in nature and function as methyl donors.

Methods Employed: A large number of analogues of S-adenosylmethionine, S-adenosylpropylamine (decarboxylated S-adenosylmethionine), S-adenosylthiomethyladenosine and S-adenosylhomocysteine will be synthesized under contract by Dr. Borchardt, and also obtained through the kind collaboration of NCI's chemotherapy synthetic program, and Prof. E. Lederer of the University of Paris. These compounds will be tested for their ability to act as substrates and/or inhibitors in a wide variety of methyltransfer and alkyltransfer reactions.

Major Findings and Proposed Course of Research: The project is just getting underway and as yet there are no findings. It is expected that it will take an additional 12 to 18 months to test the various S-adenosylmethionine analogues on a variety of enzyme systems and to decide on the future course. Several laboratories throughout the world are planing similar studies and a workshop under the aegis of EMBO has been organized in Paris for June, 1973 where there will be an opportunity for the coordination of these efforts.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 63
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Foreign Work Assignment (July 31, 1971 through September 11, 1972)

Previous Serial Number: None

Principal Investigator: G. L. Cantoni

Other Investigators: Prof. V. Monesi, M. Molinaro, S. Scarpa G. Magni and E. Scoffone

Cooperating Units: University of Rome, Italy, University of Milan, Italy, University of Padova, Italy

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Background, Objectives and Major Findings: My sabbatical year was spent in Prof. V. Monesi's laboratory at the University of Rome and in Prof. G. Magni's laboratory at the University of Milan. The choice of these two Institutes for my sabbatical period was dictated by the following considerations: In the first place, modern biology and biochemistry is moving from a period in which the heaviest concentration of effort was devoted to the elucidation of metabolic pathways and mechanisms of biosynthesis to a period in which greater emphasis will be placed on metabolic control and regulation. In this connection, it is also noteworthy that an important shif is taking place from bacteria to multicellular organisms as experimental objects. Secondly, and relatedly, a number of biological problems, notably, cellular differentiation and morphogenesis, can be studied principally if not exclusively in multicellular systems. It was my hope that the period spent in the Institutes of Profs. Monesi and Magni would serve a dual purpose. In the first place, I would become acquainted with the current research trends in chemical embryology and morphogenesis in Prof.

Monesi's laboratory since this is one of the leading institutions of this kind and, secondly, I would learn some aspects of the genetics of eukaryotes in Prof. Magni's laboratory where outstanding work on yeast genetics has been performed over the years. In both of these respects, I am pleased to report that the year has been a very profitable one for me.

It was also my hope that, from my own scientific background, I might contribute something to the work of these two laboratories. Neither Prof. Monesi's laboratory nor Prof. Magni's laboratory are primarily biochemical laboratories and, therefore, I thought that, on research problems which involve biochemical knowhow or the techniques of molecular biology, I might be able to offer some help and advice to the younger members of the laboratory.

While it is not for me to say whether this role has been adequately fulfilled, I certainly had great opportunity for exchange of ideas, discussion and scientific interchange in both laboratories, hopefully with beneficial results.

In Prof. Monesi's laboratory, I concentrated my activities as collaborator with, and consultant to, the group led by Prof. Mario Molinaro, who had spent several years as a Visiting Scientist in my own laboratory in Bethesda.

For the last few years, Molinaro and his associates have been studying the problems of muscle differentiation in cell culture. I will briefly outline the nature of the problem and indicate the lines of attack which are being pursued in his laboratory and the scope of the work.

The embryonic skeletal muscle culture system _in vitro_ has long been recognized as a potential model of cellular differentiation and therein lies its main interests. Primary cultures of cells derived from embryonic muscle of different species consists of a mixed population of at least two principal cell types with different phenotypes and biochemical properties: a) fibroblasts, and b) dividing mononucleated myoblasts. This latter group usually predominates quantitatively by three or four to one. The mononucleated myoblasts after a period of cellular division and growth make a transition into non-divided polynucleated syncythial myotubes characterized by the production of specific muscle proteins. This _in vitro_ differentiation depends on a number of conditions that have not been as yet defined with precision. In this system, proliferative and differentiative events can be clearly separated and hereby lies a unique opportunity to study the control of cell proliferation and differentiation and their interrelatedness. Although differentiation of myogenic cells is a continuous process and as such does not proceed by discrete steps, it can for purpose of discussion be separated into four phases: a preproliferation period, proliferation period, a fusion period and a post-fusion period. The most characteristic step is the transformation of mononucleated cells into multinucleated fibers.

It has been established by different workers for different systems and by Molinaro and his collaborators, using tissue cultures derived from embryonic chicks, that the multiple nuclei present in myofibers do not originate by nuclear division unaccompanied by cytoplasmic division, but rather by post-mitotic fusion of mononucleated cells. This can be demonstrated by determining the origin of the DNA in the multinucleated cells. It has been found that there is no DNA synthesis taking place in the multinucleated cells and that all the DNA contained therein is derived from DNA which has been synthesized prior to fusion. Thus, one of the signals which accompany cellular differentiation in this system is the cessation (or inhibition) of DNA synthesis and this phenomenon is accompanied (or perhaps caused) by a decline in the activity of the enzyme systems responsible for DNA synthesis. More or less, at the same time, there is an increase in the rate of synthesis of specialized muscle proteins, such as myosin, creatine kinase, myokinase and others. Just as it has not been possible to demonstrate DNA synthesis in polynucleated myofibers, it has not been possible to demonstrate synthesis of myosin in mononucleated myoblasts.

The group in Rome has spent considerable effort in trying to determine with precision the time course of myosin synthesis in tissue culture. During the months that I was in Rome, we have improved significantly on the conditions for its detection by partial protein purification of myosin followed by gel electrophoresis.

We have also spent a considerable amount of time exploring the effects of variation in the cultural condition. The culture medium in which the primary tissue explant is placed consists of a mixture of salts, amino acids, vitamins, purine, pyrimidine and nucleosides and, in addition, contains two components containing macromolecular compounds of undefined structure and whose function in the differentiation process is obscure: namely, animal serum and embryonic extracts. Generally, animal serum used in these experiments has been horse serum or fetal calf serum.

In order to elucidate the parameters involved in muscle differentiation, it would be desirable and indeed it may be necessary to understand the role of each of the components of the culture medium. This is particularly true for the animal serum and embryonic extracts, but before one is able to achieve this desirable goal it becomes necessary to establish a criterion for differentiation and this has so far proved a very difficult stumbling block.

Essentially, two approaches can be used to determine the degree of differentiation in skeletal muscle culture. The first and older approach has been the morphological one, that is an attempt to characterize the morphological appearance of the culture by microscopic examination and determination of the degree of differentiation by counting the percentage of nuclei present in multinucleated fibers and in undifferentiated mononucleated myoblasts. This technique is very tedious and time consuming, but more importantly it is fraught with pitfalls; its principal limitation is the difficulty in ascertaining whether one or more mononucleated cells adjacent to a multinucleated fiber are fused with it or still separated and maintaining their cellular integrity. Furthermore, it is rather subjective as shown by the fact that evaluation of the same microscopic field by different workers often leads to different estimates of the percentage of nuclei found in multinucleated structures. The second type of approach is a biochemical one: here one attempts to correlate cellular differentiation with one or more biochemical parameters. This obviously could be more precise, but so far it has been difficult to find a satisfactory index for the phenomenon under discussion. We have explored various approaches, such as a) measurement of the first appearance of myosin or other proteins in tissue culture, b) the correlation of incorporation of amino acids into proteins with the rate of DNA synthesis. As yet, however, these techniques have not been entirely satisfactory and the search for a differentiating index is being pursued in my own laboratory at the present time in collaboration with Dr. Scarpa, who has joined my laboratory as a Visiting Scientist. One can regard this as an extension of my year's sabbatical in Rome since it has enabled Dr. Scarpa and me to continue collaboration started in Rome and to initiate a new line of research in my own institution in Bethesda.

In spite of the lack of an entirely satisfactory criterion for the precise measurement of the course of differentiation in vitro, a number of exploratory experiments were performed aiming at the characterization of the macromolecular factors that are required for muscle differentiation in cell culture. These experiments have confirmed the conclusion of numerous other investigators that cellular differentiation requires the presence of an extracellular macromolecular factor (or factors). There is evidence that can be interpreted suggesting that this factor (or factors) is found in serum and also that a factor (or factors) is elaborated during the growth and division of myoblasts in culture. Whether these two types of factors, namely, the serum factor and the condition medium factor are one and the same or whether they are separate and distinct is not at all clear. Much further work will be necessary before a clear-cut decision is possible. From a theoretical point of view, it is attractive to think that the differentiation of myoblasts in tissue culture requires a hormone-like protein factor not dissimilar in nature from other protein factors which have been found to be involved in cellular differentiation, such as erythropoietin in the case of red blood cells, the nerve growth factor of Rita Levi-Montalcini and the epidermal growth factor discovered by Stanley Cohen.

Finally, considerable effort has also been devoted in Molinaro's group to establish if in vitro differentiation was a phenomenon involving primarily the translation of genetic information or the transcription of the information contained in the DNA genome. It might be pointed out that formally it is possible to compare cellular differentiation in eukaryotic cells with the process of enzyme induction in bacterial cells. The process of enzyme induction requires the commitment of a bacterial cell to the production of a single gene product (or a small number of gene products), that is the selection of one out of 5000 genes. Cellular differentiation likewise require the commitment of undifferentiated cells to the formation of a small number of protein with specialized biological function (hemoglobin for the red blood cell, myosin and other contractable protein for muscle, lactalbumin for the mammary gland, etc.). The selection process here may be even stricter as there are many more gene pairs in eukaryotic cells than in bacterial cells. Yet, if one of the fundamental rules of modern biology is not violated, one could expect that the same general molecular mechanisms are involved in both systems. A priori three alternative solutions to the problem of the polarization of protein biosynthesis in a very narrow range can be proposed: a) gene duplication, that is multiplication of the numbers of a specific gene in the DNA genome; b) transcriptional control, that is formation of multiple copies of messenger RNA for a particular gene product; and c) translational control, that is repeated translation of a single copy of messenger RNA. Anyone of these three solutions, singly or any number of them in combination could account for the phenomenon of enzyme induction or cellular differentiation. In bacteria, it has been conclusively demonstrated that transcriptional control alone is responsible for the phenomenon of enzyme induction. There is evidence from a few systems of eukaryotic cells undergoing differentiation that gene duplication is not detectable, and, presumably therefore, not sufficiently important to account for the phenomenon. The choice between transcription and translation control is still open, although biological bias favors the former. In the case of skeletal muscle *in vitro* the tentative conclusion can be reached from a series of experiments using inhibition of proteins or nucleic acid synthesis that differentiation in tissue cultures is not due to the operation of transcription rather than translation controls.

We have given also some thought and a little experimental effort to a different aspect of the problem. It is well established that foetal myosin and adult myosin contain a different complement of methylated amino acids and that the pattern of myosin methylation changes during the course of differentiation *in vivo*. Protein methylation involved the transfer of methyl groups from S-adenosylmethionine, a compound which I discovered and characterized in the early 1950's, to amino acids in polypeptide chains. In other words, the methylated amino acids in proteins arise from methylation of a non-methylated polypeptide chain rather than by incorporation of methylated amino acids into a growing polypeptide. There is no known mechanism for the removal of methyl groups from polypeptide chains other than proteolysis and resynthesis of a different polypeptide chain. The consequence of this argument is that the discovery that foetal myosin and adult myosin have a different complement of methylated amino acids means that they are products of two different genes and therefore that in this respect myosin synthesis resembles hemoglobin synthesis, where also one finds the synthesis of fetal homoglobin being replaced during the course of development by synthesis for adult hemoglobin. A corallary of this concept is the hypothesis that adult myosin and foetal myosin may differ by other parameters besides methylation and that such a difference might give some insight into the biological significance of myosin methylation. In this connection, it has been observed by a number of workers and substantiated in Rome that foetal myosin synthesized in tissue culture have very low ca^{++} dependent ATPase activity. It would be of considerable interest if these observations were correlated with the recently demonstrated structural differences in the amino acid sequence of foetal and adult myosin.

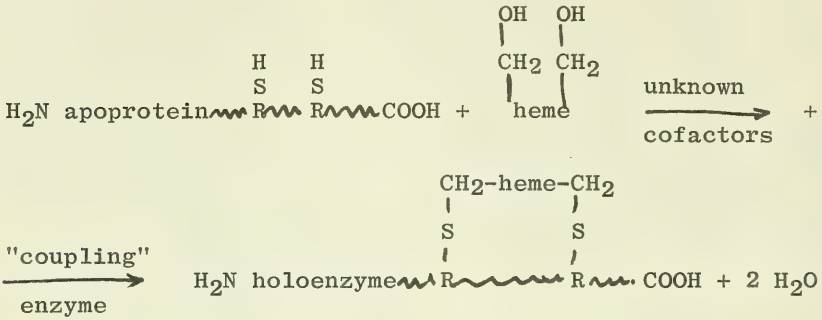
In Milan I worked on two principal projects: A) Dr. Magni and I have explored in detail various theoretical and experimental aspects of the ochre specific suppressor mutations of yeast and examined the hypothesis that the molecular basis of these mutations might be a deletion in the anticodon loop of tRNA. It should be pointed out that ochre specific suppressors exist only in yeast and the explanation of their molecular basis might have important implications for the wobble hypothesis of Crick and requires some revision thereof. After much discussion, it was decided against testing this hypothesis experimentally. While this project did not result in an experimental approach, it was nevertheless a valid intellectual exercise and contribution.

B) As a result of discussions with Prof. E. Scoffone of the University of Padova, Dr. Magni and I became engaged in a joint project with him. Dr. Scoffone has been interested for some time in the total synthesis of cytochrome c. The choice of this protein was dictated by several considerations. In the first place, it contains a single, relatively short polypeptide chain (108 amino acids). In addition, reports have appeared showing that removal and reinsertion of the heme group is possible. Substantial information is available from phylogenetic and tertiary structure studies. The biological role played by this protein is well established as is its relationship with other enzymes of the respiratory chain. Also, this synthesis can furnish the basis for a number of studies comparing genetic and chemical inducible mutations in the molecule. Last, but not least, there remains a possibility of splitting the molecule into two fragments which under appropriate conditions can be recombined with restoration of biological activity.

Dr. Scoffone and his coworkers had already synthesized by conventional procedures fragments which cover the entire sequence of the 108 amino acid residues. The problem which has concerned Profs. Scoffone and Magni and me is that of the biochemical mechanism for insertion in the heme group.

From general consideration two alternatives present themselves: a) heme group is inserted during the biosynthesis of the polypeptide chain and before its completion, b) the heme group is attached to the complete apoprotein by a specific "coupling" enzymatic reaction. The second alternative appeared more attractive, easier to test and was further elaborated and discussed. The principal problem here is the uncertainty about the tertiary conformation of the apoprotein.

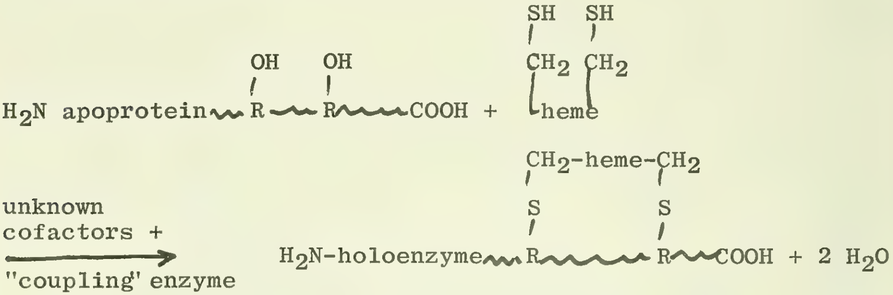
The insertion reaction catalyzed by the coupling enzyme can be schematically visualized as follows:



$$H_2N\ \text{apoprotein}\sim\overset{\substack{H\\S}}{R}\sim\overset{\substack{H\\S}}{R}\sim COOH + \overset{\substack{OH\quad OH\\|\qquad|\\CH_2\ CH_2\\|\qquad|}}{\text{heme}} \xrightarrow[\text{cofactors}]{\text{unknown}} +$$

$$\xrightarrow[\text{enzyme}]{\text{"coupling"}} H_2N\ \text{holoenzyme}\sim\overset{\substack{CH_2\text{-heme-}CH_2\\|\qquad\qquad|\\S\qquad\qquad S\\|\qquad\qquad|}}{R\sim\sim\sim R}\sim COOH + 2\ H_2O$$

If, however, as seemed probable, conformation of the apoprotein is relatively similar to that of iso-1-cytochrome c, it is very difficult to conceive how the reaction might take place because the nature of the spatial arrangements surrounding the residues 17-23 (Thr, Cys, Glu, Leu, Cyst, Hist), that provide the landmark for attachments for the heme group for the two cysteine residues, does not provide sufficient room for the heme group and the coupling enzyme. However, an alternative and equally plausible reaction mechanism for the coupling enzyme would be as follows:



$$H_2N\ \text{apoprotein}\sim\overset{\substack{OH\\|}}{R}\sim\overset{\substack{OH\\|}}{R}\sim COOH + \overset{\substack{SH\quad SH\\|\qquad|\\CH_2\ CH_2\\|\qquad|}}{\text{heme}}$$

$$\xrightarrow[\text{"coupling" enzyme}]{\text{unknown cofactors +}} H_2N\text{-holoenzyme}\sim\overset{\substack{CH_2\text{-heme-}CH_2\\|\qquad\qquad|\\S\qquad\qquad S\\|\qquad\qquad|}}{R\sim\sim\sim R}\sim COOH + 2\ H_2O$$

It will be noted that in this second scheme the amino acid serine would be found in the apoprotein instead of the amino acid cysteine in positions 19 and 22 and the SH group would be attached to the heme moiety. The substitution of serine for cysteine in the apoprotein would justify the assumption that there is a large difference in the conformation of the apoprotein compared to that of the heme protein, and this would facilitate the visualization of a biochemical reaction involving heme, coupling enzyme and apoprotein.

This hypothesis seemed attractive and we then set about to test it experimentally. The plan of the experiment is as follows: First, it was decided that it would be necessary to select auxotroph mutant yeast strains, exhibiting a strict requirement for cysteine, and this was accomplished in the Institute of Genetics, Milan, by Dr. Magni and myself.

Next, cytochrome c was to be isolated from these mutants grown with amino acids labelled with C^{14} or S^{35} serine and/or cysteine. Finally, labelled cytochrome c thus synthesized would be chemically degraded into two fragments and analysis of the fragments would permit to establish unequivocally whether the biosynthetic pathway involved incorporation of serine or cysteine at positions 19 and 23 of the cytochrome c chain. The presence of a third cysteine at position 107 serves as a convenient control for "non leakiness" of the cysteine auxotroph mutants.

Having obtained a number of cysteine auxotrophs in the Laboratory of Genetics at the University of Milan, we are in the process of performing the experiments outlined above. The answers to the experiment should be of interest whichever the outcome and provide a new insight into the mechanism of cysteine c biosynthesis.

It was a pleasure to spend a sabbatical year in the laboratories of Profs. Monesi and Magni and I want to take this opportunity to express to them and their collaborators and staff my sincere gratitude for the warm hospitality and support.

Proposed Course of Research: See Project 61.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 43
1. Laboratory of General and
   Comparative Biochemistry
2. Section on Alkaloid Biosynthesis
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Homocystinuria: Methionine Metabolism in Mammals

Previous Serial Number: Same

Principal Investigator: S. Harvey Mudd, M.D.

Other Investigators: B. W. Uhlendorf, Ph.D., J. Poole, M.D.

Cooperating Units: Laboratory of Viral Immunology, DBS
Digestive and Herediatry Disease Branch, NIAMDD

Man Years:

Total: 2-5/12
Professional: 1-1/6
Other: 1 -1/4

Project Description:

Objectives: The objectives of this project are to obtain a detailed knowledge of the biochemistry of sulfur-containing compounds in the mammalian body, with emphasis on the transsulfuration pathway. Special attention will be focused on the regulatory devices which control this area of metabolism.

Methods Employed: Most of the methods employed have been presented in detail in previous Annual Reports. In general, standard methods of enzyme purification and assay have been employed. Automated amino acid analysis has been used extensively. The techniques used for growth of cells in tissue culture are the usual ones. Nitrogen balance studies have been carried out by use of synthetic amino acid diets with appropriate supplements, generally according to the techniques used by Rose and his collaborators (see, for example, J. Biol. Chem. 216, 763 (1955)) and by Swendseid and her collaborators (see, for example, Am. J. Clin. Nutrition 16, 299 (1965)). Pyridoxic acid determinations were made according to Woodring, et al. (Clin. Chem. 10, 479 (1964)), and analyses of urinary sulfur by a previously described modification of the method of Fiske (Mudd, et al., J. Clin. Investig. 49, 1762 (1970)).

Major Findings: As in past Annual Reports, only those aspects of the overall project which have been most fruitful during the year in question will be described:

(a) We have reported before our investigations into the mechanism whereby vitamin B_6 brings about a striking restoration toward normal of the biochemical abnormalities in patients with cystathionine synthase deficiency. Results from a number of lines of experiments have led to the working hypothesis that this effect is mediated by a small increase in the residual activity of cystathionine synthase, which during treatment with B_6 may increase from a basal concentration of 1-2% of normal to a concentration of 3-4% of normal. Cystathionine synthase deficient patients who do not respond to B_6 are thought to lack even these low amounts of residual enzyme activity. Some predictions may be made on the basis of these working hypotheses. Four percent of normal cystathionine synthase should be sufficient to convert to cystathionine virtually the entire normal daily load of homocysteine (8-12 mmole in an adult taking a normal diet). One percent of this enzyme should then convert at least 2-3 mmole. The cystathionine so formed should be cleaved by cystathionase, which we have shown to be normal in these patients, thereby giving rise to an equivalent amount of cysteine. The B_6 nonresponder should not be able to form any cysteine by this means. Therefore cyst(e)ine should become an "essential" amino acid for B_6 nonresponders, but B_6-responders should show a decreased, or even negligible requirement for this amino acid. To submit these predictions to a stringent experimental test, we have initiated a series of nitrogen balance studies. To date we have confirmed previous observations that normal humans do not require dietary cyst(e)ine to remain in nitrogen balance. That is, cyst(e)ine is not normally an essential amino acid. Under our conditions, the methionine requirement appears to be significantly less than that reported by Rose for young men. Two B_6 responsive cystathionine synthase deficient patients have been studied. Neither exhibited any requirement for cyst(e)ine. Three B_6-non-responders have been studied. Two went into negative nitrogen balance as dietary cysteine was progressively decreased. From these results one may obtain an estimate of the amount of cysteine which must be formed from methionine to maintain in nitrogen balance a subject fed only methionine. Very tentatively, this amount may be placed at 2-3 mmoles. Thus, the prediction based on our working hypothesis, i.e. that the B_6 responder in his basal state should be able to form 2-3 mmoles of cysteine, appears to have been confirmed. One B_6 non-responsive patient remained in nitrogen balance even when virtually all cyst(e)ine was removed from his diet. To understand this unexpected result further studies are required. Assays of any possible residual cystathionine synthase in this patient and the kinetic properties of this enzyme should be helpful. Fibroblasts are now being grown from a skin biopsy of this patient to permit such studies.

In the course of this work we have carried out measurements of sulfur excretion under a variety of dietary conditions in both normal volunteers and patients. The results have led us to formulate the general concept of a sulfur balance, which is the analogue of the more widely recognized nitrogen balance. When our studies have been completed we should be able to determine whether or not these two balances vary in parallel, or under which conditions they diverge. At this time, it appears that under most conditions these two parameters change in parallel with the sulfur balance being a more sensitive indicator than the nitrogen balance.

It should be emphasized that these studies are currently in progress and the quantitative aspects may be modified as further results become available. Nevertheless, all results indicate strong support for the hypotheses that B_6 acts upon residual cystathionine synthase, and that a relatively small residual enzyme activity may have major physiological implications.

(b) Considerable time during the past year has been spent in preparation of manuscripts, most notably the review on "The Adenosyltransferases" listed under publications.

Scientific Significance: The approach described here of balance studies, correlated with enzyme studies, affords a means to bridge the difficult gap between in vitro enzyme assays, and physiological enzyme rates. This approach could well be extended to a variety of other situations, for example phenylketonuria, where a study of the tyrosine requirements of phenylketonuric and hyperphenylalaninemic patients might be most revealing. Determination of sulfur balances opens the way for investigation of a whole array of problems in human nutrition which have not hitherto been attacked. Finally, firm establishment of a case in which an inborn error of metabolism is treated by specific restitution of the missing enzyme activity suggests that this is a practical and humane means of therapy which should be extensively explored in a variety of human diseases. In a sense, this approach has the advantages of "genetic engineering" without some of the obvious disadvantages.

Proposed Course of Research: The dietary studies described above will be continued for a short period. However, it now appears that it will be impossible to continue these studies to their logical conclusion, or to extend them to other situations such as phenylketonuria. This is so because changes in our collaborating unit, the Digestive and Hereditary Disease Branch at this time appear to forbode a complete loss of our capability to participate in clinical studies. When Dr. J. Poole leaves on July 1, 1973, all such studies must be terminated. Further support seems not to be forthcoming, although it should be possible to complete analyses required for patient studies already completed. Thus, what now appears to be a productive and innovative approach to basic problems of human nutrition in health and disease will necessarily be phased out as our support dwindles.

Honors and Awards: None

Publications:

Mudd, S. H., The Adenosyltransferases. In The Enzymes, Volume VIII. P. D. Boyer, Ed. (Academic Press, Inc., New York, 1972).

Uhlendorf, B. W., Conerly, E. B., and Mudd, S. H., Homocystinuria. Studies in tissue culture. Ped. Research, in press.

Serial No. M - LGCB 48
1. Laboratory of General and Comparative Biochemistry
2. Section on Alkaloid Biosynthesis
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Transsulfuration in Higher Plants

Previous Serial Number: Same

Principal Investigators: John Giovanelli, Ph.D. and Anne H. Datko, Ph.D.

Other Investigators: S. Harvey Mudd, M.D. and Lowell D. Owens, Ph.D.

Cooperating Units: Agricultural Research Service, USDA

Man Years

Total: 2-1/12
Professional: 2-1/12
Other: 0

Project Description:

Objectives: Previous work has shown that extracts of spinach and other higher plants catalyze the synthesis of homocysteine (and methionine) by two main pathways - a direct sulfhydration pathway and a transulfuration pathway. The long-range objective of the project is to determine the relative physiological significance and the mechanisms of control of these pathways. The problem is being attacked along the following lines:

(1) A study of the enzymes involved in the two pathways, and the *in vivo* concentration of the substrates for these enzymes.
(2) *In vivo* evaluation of the relative physiological significance of the two pathways by use of inhibitors that specifically block one of the pathways.
(3) Physiological and biochemical studies with algae (wild type), and algal mutants in which the biosynthesis of homocysteine and methionine is affected.

Methods Employed: Most of the methods employed have been described in previous reports. Homoserine esters were isolated and purified from plant tissues by ion exchange chromatography on Dowex 1 and Dowex 50, and by paper electrophoresis and chromatography. Recovery of specific homoserine esters (e.g. O-phosphoryl-, O-acetyl-, O-malonyl-, and O-oxalyl-homoserine) was monitored by addition to the crude plant extract of the appropriate authentic compound of high specific radioactivity.

Major Findings: (1) Last year we reported the isolation from a number of plant tissues of a compound, tentatively identified as O-phosphorylhomoserine, that was capable of donating an α-aminobutyryl group in the synthesis of cystathionine by plant cystathionine γ-synthase. This α-aminobutyryl donor has now been fully characterized as O-phosphorylhomoserine by ion exchange chromatography, paper electrophoresis, and by its reaction to phosphatase and base treatment. The possibility that O-phosphorylhomoserine was derived from microbial contamination was excluded by demonstrating that the concentrations of O-phosphorylhomoserine in sterile barley seedlings and axenic cultures of Chlorella were similar to those in plant tissues grown under normal conditions. The evidence to date clearly establishes O-phosphorylhomoserine as the major compound both present in plants and capable of serving as an α-aminobutyryl donor with plant cystathionine γ-synthase. A careful study with sterile barley seedlings is now under way to determine whether α-aminobutyryl donors other than O-phosphorylhomoserine are natural components of plant tissues.

(2) Enzymic synthesis of O-phosphorylhomoserine from homoserine and ATP was demonstrated in a wide range of plant tissues. Since this enzyme is probably a control site in the synthesis of threonine and methionine in plants, it will be the subject of intense study at the earliest opportunity.

The findings listed above, together with our previously reported failure to detect the synthesis of O-succinyl-, O-acetyl-, O-oxalyl-, or O-malonyl-homoserine in plant extracts, provide strong evidence that O-phosphorylhomoserine is the physiological substrate for cystathionine synthesis in green plants. This observation is of considerable interest since in other taxa the physiological substrate is either O-succinylhomoserine (bacteria) or O-acetylhomoserine (fungi and bacteria).

(3) Considerable effort was devoted to development of sensitive and specific assays for cyst(e)ine and cystathionine in plant tissues. The assay for cyst(e)ine is a modification of the colorimetric method of Gaitonde (Biochem. J. 104: 627, 1967). The assay for cystathionine is based on a rapid exchange of radioactive cysteine with cystathionine, catalyzed by a crude preparation of Bacillus subtilis cystathionine γ-synthase. Determination of the tissue concentrations of cysteine and cystathionine by these methods will provide an additional criterion for assessing the physiological role of the transsulfuration pathway.

Scientific Significance: Discussed under individual headings above.

Proposed Course of Research: (1) Our work to date has provided strong evidence for the operation in green plants of a transsulfuration pathway in which O-phosphorylhomoserine is the major, perhaps the sole, substrate for cystathionine synthesis. However, the importance of the transsulfuration pathway relative to the direct sulfhydration pathway remains to be established. Research on this problem will continue along the following lines: (a) Determination of the substrate specificity of the enzyme(s) that catalyzes the direct sulfhydration of homoserine esters to homocysteine. (b) Determination of the concentrations of cysteine (a substrate for cystathionine synthesis), cystathionine and sulfide (a substrate for homocysteine synthesis in the direct sulfhydration pathway) in plant tissues. (c) Determination of the kinetics of incorporation of $^{35}SO_4^=$ into sulfur amino acids in the presence and absence of rhizobitoxine, which is known to inhibit plant β-cystathionase both in vitro and in vivo. (d) Combined studies on the enzymology, physiology, and genetics of the biosynthesis of sulfur amino acids in Chlorella.

(2) It is proposed to determine whether O-phosphorylhomoserine is the immediate precursor of threonine in green plants, as it is in other taxa (yeasts and bacteria). If so, the last intermediate common to the formation of methionine and threonine in green plants will be O-phosphorylhomoserine, rather than homoserine, as in other taxa. Such a change in the branch point of two pathways would indicate that the mechanisms of control of threonine and methionine biosynthesis in green plants may show interesting differences to those in other taxa.

Honors and Awards: None

Publications:

Giovanelli, J., Owens, L. D., and Mudd, S. H.: β-Cystathionase. In vivo inactivation by rhizobitoxine and role of the enzyme in methionine biosynthesis in corn seedlings. Plant Physiol. 51: 492-503, 1972.

Serial No. M-NB-1
1. Lab of Neurobiology
2.
3. Bethesda, Maryland, and
Woods Hole, Massachusetts

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Analysis of the macromolecular structure of nerve membranes during excitation

Previous Serial Number: Same

Principal Investigators: Ichiji Tasaki, M.D., Emilio Carbone, Ph.D., Kenneth Sisco, M.D. and Ronald Sandlin

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Man Years

Total:	4.6
Professional:	4.0
Other:	0.6

Project Description

Objective: During the past year we have attempted to analyze the macromolecular structure of the nerve cell membrane using four different approaches: (1) Spectral analyses of extrinsic fluorescence of the nerve membrane labelled with amino-naphthalene derivatives; (2) changes in axon birefringence associated with excitation; (3) identification of some membrane protein subunits obtained from the squid giant axon; and (4) studies of excitability, instability, and phase-transitions in squid axon membrane.

Methods Employed: Giant axons from the squid as well as the nerve trunk of crab and lobster were used to analyze spectral properties of extrinsic fluorescence of the nerve membrane labelled with amino-naphthalene derivatives. We used certain hydrophobic dyes for our staining experiments including 2-p-toluidinylnaphthalene-6-sulfonate (2,6-TNS), 1-anilinonaphthalene-8-sulfonate (1,8-ANS) and 2,6-ANS. External staining was accomplished by soaking the nerve in a solution of the dye, and internal staining was done by injecting a small volume of the dye into the axon. The stained nerve was mounted in a chamber which permitted simultaneous electrical and optical measurements. A steady low temperature was maintained by placing the nerve chamber inside a refrigerator during the experiment. The light from a 200 W xenon-mercury source was focused onto the nerve by using quartz lenses and variable diaphragms. An interference filter (365 center-band wavelength) was used to select a quasi-monochromatic beam to excite the dye molecules. Fluorescent light was collected with a hemispherical lens and detected with a photomultiplier at 90° to the excitation beam. The spectra at the peak of nerve excitation as well as in the resting state of the axon could be determined by placing an interference filter above the hemispherical lens. The interference filters ranged in wavelength of transmission from 420 nm to 580 nm. The details of this experimental technique have been explained in previous annual reports.

Birefringence techniques and flattened squid giant axons were used to investigate the conformational structure of the nerve membrane. Flattening of the axon was performed by removing the axoplasm either mechanically (using a suction pipette) or enzymatically (using a 0.05 mg/ml pronase solution) and compressing it with a small rectangular strain-free glass plate. A Leitz polarizing microscope in conjunction with a photomultiplier tube was used to measure changes in birefringence during nerve excitation. The light source was a 100 watt quartz iodine lamp. An interference filter for 540 nm wavelength was used to convert the white light from the source to quasi-monochromatic light. The amount of light from different zones of the axon was controlled by using Köhler illumination. Small changes in the photomultiplier output were amplified with a Tektronix low noise preamplifier and recorded with a CAT computer. As a rule, averaging over 1000 to 6000 trials at a repetition frequency of 25/sec was needed to obtain distinct optical signals.

An enzymatic iodination procedure was used to identify some membrane protein subunits obtained from the squid giant axon. The technique was employed to probe for accessible proteins in the outer and inner layers of the squid giant axon. The reaction mixture contained 60 μg glucose oxidase, 100 μg glucose, and 20 μCi carrier-free NaI^{125} dissolved in 1.5 ml of either filtered sea water (external medium) or internal perfusion fluid for corresponding surface layer iodination. The iodinated proteins were analyzed by disc electrophoresis in SDS according to their molecular weights.

In an attempt to investigate the phenomenon of phase transitions in the membrane macromolecules, squid giant axons (Doryteuthis bleekeri) were perfused with dilute salt solutions using an inlet and outlet cannulae technique. The potential difference across the axon membrane was measured with a glass pipette Ag-AgCl electrode filled with 0.6 M KCl solution. To reduce the outflow of the KCl solution, the pipette was filled with KCl-agar gel near the tip. The temperature of the axon was regulated by circulating the medium maintained at either low (about 4°) or high (about 20° C) temperature. To achieve uniformity of the temperature along the internally perfused axon, a baffle (made of two perforated sheets of Lucite) was constructed in the axon chamber between the inlet of the external medium and the axon. The change in temperature of the medium was very smooth and gradual taking approximately 90 sec to lower the temperature from 20° to 5° C.

Major Findings:

(1) Spectral analyses of extrinsic fluorescence of the nerve membrane labelled with amino-naphthalene derivatives: Using several fluorescent dyes the spectra of fluorescent signals were analyzed after staining the inside of

the squid giant axon and the outside of the crab nerve. In the experiments using 2,6-TNS stained nerves, the change in intensity of fluorescence (ΔI) was found to have a much narrower and sharper spectrum than the fluorescent spectrum of the light arising from labelled axons at rest. Similar results were found for 1,8-ANS and 2,6-ANS in the squid axon.

An extensive in vitro physico-chemical investigation was undertaken to explain these experimental results using 2,6-TNS. The results of other investigators were confirmed by showing that a change in solvent polarity affects both the wavelength of maximum emission and the half-bandwidths of 2,6-TNS fluorescence in a characteristic manner. This shift varies as a function of the dielectric constant and refractive index of the solvent, and, using this relationship, it was possible for us to determine the change in dipole moment of the dye molecule as a result of light excitation. The findings of our laboratory were consistent with the view that the excited dye molecule assumes a distinct second energy state in solvents with high polarity.

Extensive investigation was also made into the mechanism of fluorescent quenchers. Other factors such as temperature and viscosity were also found to affect the in vitro fluorescence of 2,6-TNS in solution.

A large number of proteins were studied which bind to 2,6-TNS and enhance its fluorescence. An attempt to simulate the resting fluorescence of 2,6-TNS in the nerve membrane was successful, but we were not able to reproduce in vitro the spectrum of ΔI (i.e., the change in fluorescence) by using various solutions of proteins and 2,6-TNS. We were however able to reconstruct the in vivo ΔI and I spectra obtained with several axons by using ethanol-water mixtures. The resting spectrum was reconstructed using a linear-combination of two ethanol-water spectra and the ΔI spectrum by taking the difference of two ethanol-water spectra. Thus we believe that although we cannot exclude the possibility that some factors other than polarity change may be involved in the production of 2,6-TNS signals in the axon membrane a change in polarity around the binding sites probably takes place. This may involve a transient increase in water molecules in the vicinity of the binding sites or movement of some polar side groups of macromolecules toward the binding sites.

(2) Changes in axon birefringence associated with excitation: A transient decrease in birefringence was recorded during nerve excitation from the center as well as the edges of flattened axons. The amplitude of the birefringence signal varied depending on whether flattening of the axon was performed after pronase digestion of the axon interior or after mechanical removal of the axoplasm. Light intensity changes (ΔI) associated with birefringence signals from axons flattened after pronase-treatment were smaller than those obtained from axons without pronase-treatment while the ratio of ΔI/I (I being the maximum light intensity observed under the crossed polar conditions at rest) was about the same for both groups of axons. In order to clarify the effect of the present pronase-treatment on the process of nerve excitation, measurement was made of the extent of membrane conductance rise at the peak of excitation as a function of time. By combining internal perfusion with

voltage-clamp technique it was possible to show that during pronase digestion the maximum value of the inward current decreases continuously. It could also be shown that the decrease in the maximum membrane conductance appears before any fall in the action potential amplitude could be recognized.

(3) Identification of some membrane protein subunits obtained from the squid giant axon: The proteins of the externally radio-iodinated axons, when subjected to SDS gel electrophoresis, displayed a wide distribution of molecular weights with a dominance at the higher molecular weights (i.e., 200,000 daltons). In contrast, proteins obtained from the internally labelled surface had a much simpler pattern with only two major peaks of radioactivity (at least 68,000 and 12,000 daltons). Depolarization of the axon with high concentrations of potassium (externally applied) appeared to decrease the efficacy of iodination of the proteins obtained from the internal surface layer.

(4) Studies of excitability, instability, and phase-transitions in squid axon membrane: Using the procedure mentioned previously, it was shown that axons internally perfused with a dilute sodium solution undergo an abrupt transition from a resting to a depolarized state on addition of KCl to an external medium containing $CaCl_2$. Under internal perfusion with a dilute solution of Na or Cs salt, it was possible to induce abrupt transitions between the two (i.e., resting and depolarized) states of the membrane by changing the temperature. "Giant fluctuations" in the state of the axon membrane were demonstrated at and near the critical points of the axon membrane.

Significance: The spectral analyses of the fluorescence of 2,6-TNS, 2,6-ANS and 1,8-ANS bound to the nerve membrane at rest and at the peak of nerve excitation give us a new and important piece of information about the local environment surrounding the dye molecules. We now have strong evidence that during nerve excitation the fluorochrome is monitoring an increase of solvent-polarity in its microenvironment, confirming our previous investigation that the nerve membrane undergoes a transition from a hydrophobic to a hydrophilic state. It is, however, very difficult at this stage to distinguish a local increase in water molecules from a single movement of some polar side-group of membrane macromolecules toward the binding sites. The interpretation of the in vivo data was possible only after a rather extensive and systematic physico-chemical study of the properties of the dye molecules.

Birefringence studies of flattened axons during excitation indicated the involvement of a longitudinally oriented crystal-like structure at or near the inner surface of the axon membrane in the process of nerve excitation. This finding confirms our previous observation of the existence of a highly oriented longitudinal structure obtained by the use of the fluorescence polarization technique with 2,6-TNS. The consistently smaller birefringence signals obtained from axons treated enzymatically led us to a closer investigation of this phenomena. We observed that enzymatic digestion of proteins in and near the axon membrane reduces either directly or indirectly the extent of structural change associated with nerve excitation.

Studies of instability and temperature effects on membrane potential in perfused squid axon supported the view that the abrupt change in the conductance and membrane potential is an electrochemical manifestation of a phase transition of the membrane macromolecules. Existence of "giant fluctuations" in the state of the axon membrane at and near the critical points of the axonal membrane is consistent with theoretical consideration of equilibrium and non-equilibrium thermodynamics.

Proposed Course: We intend to pursue our fluorescence studies to elucidate the precise structures involved in the production of fluorescent signals. More emphasis will be given to spectral and polarization studies of the axonal membrane labelled externally with aminonaphthalene derivatives. Measurements of fluorescence lifetime of the dye molecules bound to the axon membrane will be initiated. As always, considerable emphasis will be placed on the study of physico-chemical parameters influencing the fluorescence of probe molecules. We will also investigate in further detail the effect of enzymes and different internal perfusion solutions on birefringence of squid giant axons. Resting conductance and conductance change measurements during excitation will be performed under various electrophysiological conditions.

Honors and Awards:

Dr. Tasaki was invited to present a paper at the Fourth Biophysics Congress which was held in Moscow, Russia. In addition he was asked to present papers at the Conference on Macromolecular Matrices and Carriers and the Symposium on Conformation Structure of Polymers, both held in Prague, Czechoslovakia.

Dr. Tasaki was a participant in a conference on "The mechanism of energy transduction in biological systems," sponsored by the New York Academy of Sciences.

Publications:

Hallett, M. and Carbone, E.: Studies of calcium influx into squid giant axons with aequorin. J. Cell. Physiol. 80: 219-226, 1972.

Inoue, I., Kobatake, Y. and Tasaki, I.: Excitability, instability and phase transitions in squid axon membrane under internal perfusion with dilute salt solutions. Biochim. Biophys. Acta 307: 471-477, 1973.

Tasaki, I. and Hallett, M.: Bioenergetics of nerve excitation. Bioenergetics 3: 65-79, 1972.

Tasaki, I., Hallett, M. and Carbone, E.: Further studies of nerve membranes labelled with fluorescent probes. J. Memb. Biol. 11: 353-376, 1973.

Serial No. M-NB-2
1. Lab of Neurobiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Sensory-motor integration in the primate visual system

Previous Serial Number: Same

Principal Investigators: Robert H. Wurtz, Ph.D., David L. Robinson, Ph.D., Charles W. Mohler, Ph.D. and Charlene D. Jarvis, Ph.D.

Man Years

Total:	6.5
Professional:	4.0
Other:	2.5

Project Description

Objective: We have studied three areas of the brain of the monkey in the past year in order to determine the contribution of each area to the processing of visual information and the production of behavior. Our goal is a better understanding of vision and visually guided behavior.

Methods Employed: The discharge of single brain cells has been recorded in awake monkeys (*Macaca mulatta*) trained to do several visually related tasks. The monkeys sat in a chair facing a screen and fixated on a point of light for several seconds in order to obtain a reward. During this fixation period, a second spot of light was projected onto the screen and used to determine the area of the visual field in which the spot modified the discharge of the cell (the visual receptive field of the cell). Rapid eye movements were elicited by turning off one fixation point and turning on another point thereby inducing an eye movement from one spot to another. Slow tracking eye movements were produced by moving the spot slowly across the screen.

In the past year a substantial amount of the time of Drs. Wurtz and Mohler has been devoted to converting one of two recording systems for these experiments to a computer controlled system. This system has been operable throughout the year, but has required revisions to obtain maximum control of the experiment. The computer now controls on-line the monkey's behavior, and presents visual stimuli, displays the single cell discharge in relation to the behavior or stimuli, displays and recognizes the occurrence of eye movements, photographs these displays, and stores the data on digital tape. A set of programs stored in the computer permits us to ask many different questions of each brain cell, provides an immediate answer, and allows us to study more thoroughly a cell before it is lost.

Major Findings: Three general investigations on visual-motor integration have been carried out in the last year: the visual function of the "frontal eye fields", the generality of the "attention" effect found previously in the superior colliculus, and the integration of head and eye movement control in the superior colliculus.

Stimulation of one area of the frontal cortex has been known to produce eye movements for over a century. These "frontal eye fields" have been assumed to be a motor area which initiates eye movements comparable to the way that the precentral gyrus region initiates skeletal muscle movements. However, none of the cells in the frontal eye fields discharge before eye movements. We thought that because this frontal eye field area receives input from the two main visual areas, occipital cortex and the superior colliculus of the midbrain, the region might have visual functions. For 137 cells studied in the frontal eye fields of two monkeys this has indeed been the case; nearly half of the cells had clearly defined visual receptive fields. Cells responded to small stationary spots of light over large areas of the visual field -- from an area 40° of arc in diameter up to the entire contralateral visual field. For some cells the visual receptive fields also overlapped midline by 10° to 20° into the ipsilateral visual field. Any shape of the stimulus was adequate for these cells and no particular direction or speed of movement was required. These cells are therefore similar to the cells found in the superior colliculus and very dissimilar to those found in area 17 of visual cortex. Another 23% of the cells in the frontal eye field discharged only after eye movements. Some of these eye movement related cells did not discharge after eye movements in darkness, but appeared to discharge only after visually guided eye movements.

In a second phase of our work we extended our previous finding that some cells in the brain responded vigorously when a monkey paid "attention" to the visual stimulus. These cells in the superior colliculus responded to visual stimuli, but the response was enhanced when the monkey used the stimulus as the target for a saccadic eye movement and thereby attended to the spot of light. We have now determined whether this attention effect is also present in the primary cortical visual area (area 17) and in the frontal cortex (frontal eye fields). In frontal eye fields the enhancement is present in about half of the cells studied, a ratio about the same as that seen in the superior colliculus. In area 17, however, no such enhancement has been found.

Our third line of investigation has considered the role of the superior colliculus in eye-head coordination. Previous studies in this laboratory have shown that cells in the intermediate layers of the superior colliculus discharge before saccadic eye movements made to areas in the contralateral visual field. However, these experiments were conducted using monkeys which had their heads restrained. Since shifts in gaze frequently involve coordinated head and eye movements, we conducted experiments to determine if some of the cells in the intermediate layers of the superior colliculus might be better related to head movements or coordinated head and eye movements than to eye movements alone. Monkeys were fitted with a light weight head holder which allowed them to make horizontal head movements as well as eye movements in order to fixate a spot of light. While the animals performed this task we recorded from cells in the superior colliculus. All of the cells which we recorded in the intermediate layers of the colliculus discharged before contralateral saccadic eye movements and these discharges were closely synchronized with the eye movement whereas they were not synchronized with head movements. We never encountered cells in the superior colliculus which discharged in relation to head movement or head position.

Significance: Our first series of experiments show that the frontal eye fields clearly are not motor areas for eye movement as is the commonly held view. Instead this area may be appropriately referred to as a frontal cortex visual area. The coexistence of cells with visual receptive fields and cells responding after saccades suggests that one function of this frontal area may be related to an interaction between visual and eye movement information. Stimulation of this area may produce eye movements by affecting activity of these visual cells which in turn present visual data to the brainstem oculomotor centers of the brain.

The second series of experiments found that the visual response of cells in the frontal eye field greatly increased when the monkey attended to or used the visual stimulus activating the cells. Thus both the superior colliculus in the brainstem and an area of frontal cortex show this attention effect. Selection of some stimuli as opposed to other stimuli is a constant process in the normal behavior of man and other animals. The enhancement of cell responses we observed indicates that this selection process is operating in the brainstem and frontal cortex and provides an initial understanding of the brain mechanisms involved in the selection process. Failure to find such enhancement in area 17 of visual cortex indicates that such selection does not occur in the primary sensory cortex for vision but instead occurs further on in the sensory system.

Finally, the observation that colliculus cells are related to eye but not head movement clarifies points of uncertainty both for the colliculus and of eye-head coordination. Anatomical and physiological studies, particularly in cats, had suggested that the superior colliculus was important in coordinating eye and head movements. From our study of cell responses, it is clear that the superior colliculus of the monkey does not integrate visual information for both head and eye movement but rather for eye movement alone. Head and eye movement must be integrated at later stages in the nervous system.

Proposed Course: We will study further the effect of "attention" on the response of cells in the brain. We have so far observed this "attention" effect only when the monkey responded to the stimulus by making an eye movement; we next want to ask whether these cells will show this enhanced cell discharge when the monkey uses the visual information in other ways such as with a hand movement but with no eye movement at all. Along a somewhat different line, we would also like to see which areas of the brain are necessary for the accurate guidance of eye movements. We previously found that destruction of the superior colliculus did not interfere with the guidance of saccadic eye movements. We plan to damage small areas of visual cortex and frontal eye fields to see the contribution of these areas to visual guidance of saccadic eye movements.

Honors and Awards:

Dr. Wurtz was invited by the Neurosciences Research Program to participate in a Work Session on, "Sensory-motor function of midbrain tectum."

Publications:

Goldberg, M.E. and Wurtz, R.H.: Activity of superior colliculus in behaving monkey. I. Visual receptive fields of single neurons. J. Neurophysiol. 35: 542-559, 1972.

Goldberg, M.E. and Wurtz, R.H.: Activity of superior colliculus in behaving monkey. II. Effect of attention on neuronalresponses. J. Neurophysiol. 35: 560-574, 1972.

Wurtz, R.H. and Goldberg, M.E.: Activity of superior colliculus in behaving monkey. III. Cells discharging before eye movements. J. Neurophysiol. 35: 575-586, 1972.

Wurtz, R.H. and Goldberg, M.E.: Activity of superior colliculus in behaving monkey. IV. Effects of lesions on eye movements. J. Neurophysiol. 35: 587-596, 1972.

Wurtz, R.H. and Goldberg, M.E.: The primate superior colliculus and the shift of visual attention. Investigative Ophthalmology 11: 441-450, 1972.

Serial No. M-NB-3
1. Lab of Neurobiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physicochemical characterization of biological membranes

Previous Serial Number: M-NB-1

Principal Investigator: Allan S. Schneider, Ph.D.

Man Years

Total:	2.1
Professional:	1.0
Other:	1.1

Project Description

Objectives: The objectives of this project are:

1. Analysis of membrane-water interactions
2. Location of calcium binding sites in membranes
3. Conformational analysis of membrane and storage proteins of catecholamine storage vesicles (chromaffin granules).

Methods Employed: Membrane hydration was studied using hydrophobic fluorescent probes (naphthalene sulfonates) and gravimetric methods. Membrane-calcium interactions were analyzed via a calcium-sensitive fluorescent probe, namely the tetracycline antibiotics. Membrane protein conformation was determined by circular dichroism and absorption spectroscopy.

Major Findings:

1. Membrane hydration: The fluorescence spectra of hydrophobic probe dyes (ANS, TNS) in the presence of red cell membranes were found to undergo changes with membrane hydration characteristic of tightly bound water at low hydrations and loosely-held, fluid water at high-hydrations. The water content in the membranes and the energetics of water binding have been previously determined.

2. Membrane-calcium interactions: Chlortetracycline (CTC) fluorescence has been shown to be sensitive to membrane associated calcium both in nerve and red cell membranes. Experiments designed to locate the CTC-calcium-membrane binding sites implicate the phosphate headgroups of the membrane phospholipids.

3. Protein conformation of membrane and contents of catecholamine storing vesicles (chromaffin granules): A combined program of experiment and calculational analysis of the UV absorption and circular dichroism (CD) spectra of catecholamine storage vesicles has been completed. Circularly polarized light scattering effects of the CD spectra have been resolved. The protein conformation (2° structure) of the chromaffin granule membrane and storage proteins have been determined before and after release.

Significance: The structure of biological membranes and the interactions of membrane components lie at the heart of almost every aspect of cellular physiology. In the nervous system it is the axonal membrane which conducts the nerve impulse and the synaptic membranes which are critically involved in transmission. It is very likely that the molecular events controlling nerve excitation and transmission will not be clearly understood until the structure of the cell membrane is solved for simpler cells whose membranes can be isolated in a pure and functioning form. The significance of our work on model membrane systems thus follows:

1. The membrane-hydration studies indicate that upon dehydration of membrane-dye samples, changes occur in the fluorescence emission spectrum which are characteristic of increased membrane hydrophobicity and loss of mobility of the water molecules. These results are consistent with postulated hydrophobic-hydrophilic changes associated with nerve excitation.

2. The location of calcium binding sites at membrane lipid-phosphate head-groups should prove useful in monitoring calcium related membrane functions as well as in observing mobility of membrane phospholipids.

3. The determination of protein conformation of the chromaffin granule membrane and storage proteins both before and after release are relevant to the mechanism of neurosecretion and hormone release. Our findings that the storage proteins do not change conformation after release is significant to the existence of a possible storage complex in the mechanism of catecholamine uptake and storage.

Proposed Course: The project will be terminated.

Honors and Awards:

Dr. Schneider was an invited lecturer and consultant at the Weizmann Institute of Science, Rehovot, Israel. He was also an invited participant at the California Membrane Conference held in Squaw Valley, California, and a lecturer at the University of California, San Francisco Medical Center.

Publications:

Hallett, M., Schneider, A.S. and Carbone, E.: Tetracycline fluorescence as a calcium probe for nerve membrane with some model studies using erythrocyte ghosts. J. Memb. Biol. 10: 31-44, 1972.

Schneider, A.S.: Analysis of optical activity spectra of turbid biological suspensions. In Hirs, C.H.W. and Timasheff, S.N. (Eds.): Methods in Enzymology: Enzyme Structure. New York, Academic Press, 1973. Vol. 27(D).

Schneider, M-J. T. and Schneider, A.S.: Water in biological membranes: Adsorption isotherms and circular dichroism as a function of hydration. J. Memb. Biol. 9: 127-140, 1972.

Serial No. M-NC-1
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Conversion of Phenylalanine to Tyrosine

Previous Serial Number: M-NC-1

Principal Investigator: Seymour Kaufman

Other Investigators: S. Milstien, Daniel Fisher,
J.-P. Abita

Man Years:

Total	:	3.4
Professional	:	2.4
Other	:	1.0

Project Description:

Objectives: The oxidation of foodstuffs, which ultimately supplies the energy for all cellular functions, can be effected by two completely different processes. Organic compounds can either be oxidized by dehydrogenation, the removal of hydrogen, or by oxygenation, the addition of oxygen to the substrate molecule. The first type of reaction predominates in carbohydrate and fat metabolism. The mechanism of this kind of oxidation has been the subject of investigation for 50 years and is now quite well understood. Oxygenation reactions are encountered mainly in the cellular catabolism of ring compounds such as aromatic and heterocyclic amino acids, hormones and drugs. In contrast to hydrogenations, the mechanism of oxygenation reactions has remained obscure. The conversion of phenylalanine to tyrosine is an oxidation reaction of this type and the goal of this study is to gain insight into the mechanism of this kind of oxidation reaction.

Methods Employed: Standard methods of protein purification, including salt and ethanol fractionation, ion-exchange chromatography and sephadex-gel filtration, have been used. The following methods of protein analysis have been used to characterize the hydroxylase: Sucrose-density ultracentrifugation and polyacrylamide gel electrophoresis.

Major Findings: 1. The pure hydroxylase from rat liver has been found to be markedly activated by limited proteolysis. During this process of digestion, the size of the enzyme molecule is reduced by about 30%. Limited proteolysis produces a hydroxylase that is indistinguishable in its kinetic properties from the hydroxylase that is activated by phospholipids (reported last year).

2. One of the properties of the hydroxylase that is altered by either proteolysis or phospholipids is its amino acid substrate specificity. After either treatment, the enzyme is able to utilize its normal product, tyrosine, as a substrate. In the presence of tyrosine, the altered enzyme catalyzes a new reaction, i.e., the tyrosine-dependent oxidation of tetrahydrobiopterin to dihydrobiopterin and H_2O_2.

3. Using the activation by phospholipids to increase the activity of the hydroxylase, we have detected, for the first time, some hydroxylase activity in a liver biopsy sample from a patient with phenylketonuria (PKU). The activity is so low (0.27% of normal) that it could not have been detected without the more sensitive assay that was made possible by our previous finding of activation by phospholipids. Some of the kinetic properties of the mutant enzyme are different from that of the normal enzyme.

4. Phenylalanine hydroxylase has been partially purified from human liver. Whereas most of the properties of the human enzyme are similar to those of the rat liver enzyme, there are some important differences, e.g., in the presence of lysolecithin (an activating phospholipid) and tetrahydrobiopterin, the K_m of the human liver enzyme for phenylalanine is about 1/5 that of the rat liver enzyme. It is of interest that this value for the K_m is about the same as the normal serum concentration of phenylalanine in humans.

Scientific Significance: The finding that phenylalanine hydroxylase can be activated by partial proteolysis to give a molecule with the same kinetic properties as the phospholipid-activated hydroxylase should facilitate a study of the structural basis of the activation. It also provides another mechanism by which the *in vivo* regulation of the hydroxylase may be achieved. It is possible, for example, that the stability of lysosomes in the liver cell is a determinant of the activity of the hydroxylase. Agents that can labilize the lysosomal membrane, and thereby release intracellular proteases, might be capable of activating the hydroxylase.

The detection of very low levels of phenylalanine hydroxylase in a liver sample from a PKU patient answers several important questions about the disease.

1. PKU is not caused by a deletion mutation since a gene product has now been detected. The disease is therefore probably caused by a mutation that leads to an amino acid substitution in the enzyme. The mutant enzyme has low hydroxylase activity and is kinetically-distinguishable from the normal enzyme.

2. The low hydroxylase activity can be used as an assay for the eventual isolation of the mutant enzyme. To accomplish this, however, one would need a moderate amount of fresh liver from a patient with PKU.

3. The finding that there is some, albeit a very small amount, of the hydroxylase makes it logical, for the first time, to attempt to enhance this activity *in vivo*. If such attempts were successful, an alternate form of therapy for PKU might be developed. This is one reason why the activation of the normal enzyme by phospholipids and by proteases (see above) is significant.

Proposed Course: Further studies on the mechanism and regulatory properties of the enzyme will be carried out to explore possibilities for enhancing the *in vivo* activity of the enzyme.

Honors and Awards: None

Publications:

Fisher, D. B., Kirkwood, R. and Kaufman, S.: Rat liver phenylalanine hydroxylase, an iron enzyme. *J. Biol. Chem.* 247: 5161-5167, 1972.

Craine, J. E., Hall, E. S. and Kaufman, S.: The isolation and characterization of dihydropteridine reductase from sheep liver. *J. Biol. Chem.* 247: 6082-6091, 1972.

Friedman, P. A., Kaufman, S. and Kang, E. S.: Nature of the molecular defect in phenylketonuria and hyperphenylalaninemia. *Nature* 240: 157-159, 1972.

Friedman, P. A., Fisher, D. B., Kang, E. S. and Kaufman, S.: Detection of hepatic 4-phenylalanine hydroxylase in classical phenylketonuria. *Proc. Nat. Acad. Sci., U.S.A.* 70: 552-556, 1973.

Friedman, P. A. and Kaufman, S.: Some characteristics of partially purified human liver phenylalanine hydroxylase. Biochim. Biophys. Acta 293: 56-61, 1973.

Fisher, D. B. and Kaufman, S.: Tetrahydropterin oxidation without hydroxylation catalyzed by rat liver phenylalanine hydroxylase. J. Biol. Chem. 1973, in press.

Fisher, D. B. and Kaufman, S. The stimulation of rat liver phenylalanine hydroxylase by lysolecithin and α-chymotrypsin. J. Biol. Chem. 1973, in press.

Huang, C. Y. and Kaufman, S.: Studies on the mechanisms of action of phenylalanine hydroxylase and its protein stimulator. J. Biol. Chem. 1973, in press.

Huang, C. Y., Max, E. E. and Kaufman, S.: Purification and characterization of phenylalanine hydroxylase stimulating protein from rat liver. J. Biol. Chem. 1973, in press.

Serial No. M-NC-2
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biosynthesis of Catecholamines

Previous Serial Number: M-NC-2

Principal Investigator: Thomas A. Lloyd

Other Investigators: Seymour Kaufman, Robert Hoeldtke

Man Years

Total : 1.7
Professional: 1.7
Other : 0

Project Description:

Objectives: These investigations are a continuation of previous work in this laboratory concerning the biosynthesis and regulation of catecholamines. Since tyrosine hydroxylase has been demonstrated to be the rate limiting step in the biosynthesis of catecholamines, present goals include: A further purification of tyrosine hydroxylase from mammalian central nervous system, isolation and characterization of the brain tyrosine hydroxylase cofactor, and analysis of the regulation and turnover of mammalian tyrosine hydroxylase under physiological and pharmacological manipulation.

Methods Employed: The production of specific tyrosine hydroxylase-directed antibodies has been achieved through techniques similar to those used in this laboratory for the production of specific phenylalanine hydroxylase-directed antibodies. A partial purification of bovine caudate tyrosine hydroxylase involving salt fractionations and chromatography of the enzyme on an affinity column has yielded a reproducible and stable preparation which has been purified 30 times over a tissue extract.

Major Findings: Production of antibodies to bovine adrenal tyrosine hydroxylase; cross-reactivity studies with other pterin-dependent hydroxylases.

Based on experience with other enzymes, it would seem that the availability of specific antibodies to tyrosine hydroxylase would be an invaluable tool in the further studies of the physiological regulation of the enzyme and the related question of its intracellular localization. With this end in mind, we have, by disc-gel electrophoresis, prepared highly purified bovine adrenal tyrosine hydroxylase and used this preparation to induce the formation of specific antibodies in sheep to the hydroxylase. Enzyme inhibition studies and agar double-immunodiffusion reactions revealed extensive cross-reactivity between the antiserum and tyrosine hydroxylases from bovine, rat, monkey, and rabbit adrenal glands as well as from bovine caudate and human pheochromocytoma. The specificity of the antiserum was demonstrated by the production of only a single precipitin line to a bovine adrenal gland extract and by the lack of inhibition of phenylalanine hydroxylase, tryptophan hydroxylase, dopamine β-hydroxylase, and mouse melanoma tyrosinase.

Scientific Significance: The present finding that the chymotrypsin-solubilized and the soluble bovine adrenal tyrosine hydroxylases are immunologically-indistinguishable, demonstrates that the protease treatment not only does not significantly change the catalytic properties of the enzyme, but that its antigenic determinants are unaltered. Our previous study demonstrated that the highly specific antiserum to pure rat liver phenylalanine hydroxylase did manifest some cross-reactivity towards tyrosine hydroxylases from both bovine adrenal medulla and rat brain. In contrast, our present study has shown that tyrosine hydroxylase-directed antibody has absolutely no cross-reactivity towards rat liver phenylalanine hydroxylase.

We included tyrosinase in our study of the specificity of the antiserum because it is known that the first step in the conversion of tyrosine to melanin catalyzed by tyrosinase is the hydroxylation of tyrosine to dopa, i.e., tyrosinase has a tyrosine hydroxylase-like activity. This partial overlap of catalytic activities between tyrosinase and tyrosine hydroxylase suggests that the two enzymes might have evolved from a common protein precursor. Our negative cross-reactivity results indicate that even if the two enzymes were once related, they are now structurally-dissimilar.

Proposed Course: The specific tyrosine hydroxylase-directed antiserum should prove valuable in carrying out both regional and intra-cellular localization studies of tyrosine hydroxylase. In view of its widely-held role as the rate limiting enzyme in the biosynthesis of norepinephrine, we believe that the careful use of a specific antiserum to tyrosine hydroxylase should provide the first truly quantitative method for studying the turnover time of this enzyme under normal conditions and under physiological and pharmacological manipulation. Preliminary investigations in this laboratory have also shown that the antiserum can be used for purifying diverse tyrosine hydroxylases.

Honors and Awards: None

Publications:

Friedman, P. A., Lloyd, T. and Kaufman, S.: Production of antibodies to rat liver phenylalanine hydroxylase; cross-reactivity with other pterin-dependent hydroxylases. Mol. Pharmacol. 8: 501-510, 1972.

Lloyd, T. and Kaufman, S.: Production of antibodies to bovine adrenal tyrosine hydroxylase; cross-reactivity studies with other pterin-dependent hydroxylases. Mol. Pharmacol. 1973, in press.

Serial No. M-NC-4
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Biochemical Basis of Skeletal Muscle Hypertrophy

Previous Serial Number: M-NC-4

Principal Investigator: Seymour Kaufman

Other Investigator: Richard Bailyn

Man Years

Total : 2.1
Professional: 1.3
Other : 0.8

Project Description:

Objectives: The objective of this project is unchanged: The elucidation of the biochemical correlates of muscle cell hypertrophy.

Methods Employed: We are using an operation to produce work-induced hypertrophy of skeletal muscle *in vivo* as previously described. The biochemical fractions of interest are labeled isotopically. RNA is separated by a modified Schmidt-Tannhauser procedure and phospholipids and their precursors by the Folch procedure.

Major Findings: Our previous work established that skeletal muscle hypertrophy induced by cutting the tendons of the synergistic muscle leads to a proliferation of fibroblasts in the hypertrophied muscle. We have previously shown that these cells, which are believed to be precursors of connective tissue cells, are the site of enhanced RNA synthesis that is characteristic of hypertrophied muscle. We have attempted to examine the possibility that fibroblast proliferation is an integral step in the onset of hypertrophy.

Two approaches were used, both based on the fact that collagen biosynthesis is a characteristic reaction of fibroblasts. We have found that neither indomethacin administration nor vitamin C deficiency, two interventions that are known to interfere with collagen metabolism, inhibited the development of hypertrophy.

Another aspect of the problem that was studied is the type of myosin that is synthesized during skeletal muscle hypertrophy. It was found that by both kinetic and structural criteria, the newly synthesized myosin in hypertrophied muscle was indistinguishable from the myosin in the control, unhypertrophied muscle.

Scientific Significance: The results of the vitamin C-deficiency experiments are still preliminary. If they are substantiated by future work, these results will disprove any model for hypertrophy that invokes fibroblast proliferation (and the accompanying enhancement of collagen synthesis) as early steps in the development of hypertrophy.

The finding that the myosin that is synthesized in muscles undergoing hypertrophy is indistinguishable from that present in control muscle does not support a recent proposal that the synthesis of fetal myosin may be renewed during muscle hypertrophy. Our findings also show that the control of myosin synthesis by muscle work is different from the control of myosin synthesis by neural activity. In the latter case, the kind of myosin synthesized is determined by the type of nerve that innervates the muscle.

Proposed Course: Experiments with larger numbers of scorbutic animals will be set up to establish whether the preliminary conclusion, *i.e.*, that collagen biosynthesis is not a requisite early step for muscle hypertrophy, is valid.

Honors and Awards: None

Publications:

Jablecki, C. and Kaufman, S.: Myosin ATPase activity during work-induced growth of slow and fast skeletal muscle in the normal rat. *J. Biol. Chem.* 248, 1056-1062, 1973.

Serial No. M-NC-5
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Process of Lysogeny

Previous Serial Number: M-NC-5

Principal Investigator: Howard A. Nash

Man Years

Total	2
Professional	1
Other	1

Project Description:

Objectives: The project is a continuing study of the processes involved in the insertion of viral DNA into the host genome. An elucidation, at the level of biochemistry and molecular biology, of the steps involved in DNA-DNA recombination is the overall goal.

Methods Employed: Microbiological techniques for isolation, growth, genetic characterization of bacteriophages; radioactive and/or density labeling procedures for bacteriophage; buoyant density and sedimentation analysis using density gradients in a high speed preparative ultracentrifuge; polyacrylamide gel electrophoresis; chromatographic column separations.

Major Findings: Bacteriophage λ linearly inserts its DNA into that of the host bacterium by a recombination between non-identical DNA sequences on host and phage chromosomes. We have succeeded in constructing a bacteriophage which contains both of these sequences, called attachment sites, on a single phage genome. This mixed phage was created by an extremely rare illegitimate recombination event and was selected by a combination of genetic and physical means. The occurrence of two recombining sites on the same molecule creates the possibility of intramolecular recombination and this possibility is realized in this system. Intramolecular recombination is found to be extremely efficient by comparison with ordinary, intermolecular recombination and leads to overall recombinant phage yields approaching 100%.

Recombination between integration sites in this system has been shown to display many of the features known to occur in normal integration. The recombination event occurs precisely at the attachment sites, causing deletion of only the genetic information which maps between them. A uniform population of smaller phage genomes is created and are characterized by hybrid recombination sites identical in properties to that of one of the inserted prophage attachment sites. The recombination event takes place under the direction of the product of the *int* gene. *Int* is the only gene known to be involved in *normal* *integration*; several other genes involved in other recombinations are neither required nor used by double attachment phage.

The existence of a system that undergoes integrative recombination at high efficiency makes for convenience and accuracy in the study of the physiology of integration. We have shown that integrative recombination, in marked contrast to the reverse (excisive) reaction, is highly temperature sensitive, with recombinant yields falling 100-fold between 30° and 45°. *Int* gene product appears to be used in more than catalytic amounts, since the yield of recombinants varies strongly with the dosage of *int* gene product supplied. Finally, we have used these *double* attachment site phages to demonstrate and investigate the production/utilization of the *int* gene products coded for by a prophage which is maintained *in* the repressed state. With this assay we have discovered that a gene, *xis*, involved in the reverse of the integration process, *produces* a potent inhibitor of this production/utilization. Efficient recombination using the *int* gene product of a repressed prophage only occurs when *the* *xis* function is removed by mutation. In mixed infection with *wild* type and *xis* mutant double attachment phage, production/utilization *of* *int* from repressed prophage is inhibited for both phage *types*. Thus, the product of the *xis* gene acts as a classical diffusible repressor/*inhibitor*.

The high efficiency of site-specific integrative recombination in double attachment site phages has been exploited to establish the first *in vitro* assay of integrative recombinations. Cells *infected with* these phage for various times are broken open; nucleic acids are extracted and assayed for the fraction of recombinant phage DNA by tranfection of spheroplasts and physical analysis of the resulting phage burst. Inhibitors of macromolecular metabolism have been used to show: a) the product of the *xis* gene is an inhibitor of integrative recombination, b) *there* is no requirement for transcription or translation of a phage DNA molecule in order to render it a suitable substrate for integrative recombination, and c)transcription of phage DNA renders it a poor substrate but this effect is fully reversible.

Scientific Significance: Genetic recombination is the last fundamental macromolecular transformation of molecular biology to be studied at the biochemical level. Much of the reason for this has been the absence of *in vitro* study systems for the production and detection of recombinants. This work advances one segment of genetic recombination studies to the threshold of a fully *in vitro* analysis. In addition, the present results add to the small body of knowledge which relates to the molecular events in recombination. Of particular interest are the interference with integrative recombination shown both by *xis* gene product and transcription of the recombining molecule. These factors not only help to define the nature of the recombination substrate, but additionally must play a role in determining the timing of the integrative event in normal infection.

Proposed Course: We will continue to emphasize the biochemical analysis of recombination. Directions taken will include extension of the *in vitro* analyses to include recombination by open cell systems, examination of additional phage and bacterial genes for involvement in integration, investigation of the nature of the inhibition of integration by *xis* gene product, and extension of our *in vitro* methodology to generalized phage and bacterial recombination.

Honors and Awards: None

Publications:

Hidalgo, C. and Nash, H. A.: Methylation pattern of lambda deoxyribonucleic acid. *J. Virology* 10: 937-942, 1972

Serial No. M-NC-6
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physicochemical Investigations of Biofunctional Structures of Glycosaminoglycans, Glycolipids and Complex Polysaccharides

Previous Serial Number: M-NC-6

Principal Investigator: Audrey Larack Stone

Other Investigators: a) E. J. Smith

Cooperating Units: US Department of Agriculture

Man Years

Total : 0.5
Professional: 0.5
Other : 0

Project Description:

Physicochemical measurement of optical conformations of complex polysaccharides and the glycosaminoglycans (GAG, mucopolysaccharides); determination of the molecular basis for the formation and maintenance of inter- and intra-molecular structures known to be involved in the rapid transformation of simple cations (and biogenic amines) from the bound to the active form; and measurement of conformation of intermediates in enzyme reactions in ganglioside synthesis.

a) Measurement of primary and secondary structure of bacterial exopolysaccharides (related to mammalian intercellular matter such as hyaluronic acid) using optical methods.

Objectives: To elucidate the particulars of the various molecular conformations of GAG by means of their recently discovered (M-NC-21, 1967) ultraviolet amide transition bands and the special optical effects of their dye complexes (M-NC-21, 1965) with the view towards developing a sensitive method of identification, and a molecular basis for biological activities associated with interstitial matrices such as the greater neuronal membrane. To demonstrate specifics of and specificities of molecular conformations in solution of variously related complex polysaccharides (known to form helical structures in oriented fibers), using techniques developed earlier in this project.

Major Findings: Standard GAG exhibit particular CD in the spectral region of the $n-\pi$ and $\pi-\pi$ amide transitions (see M-NC-6, 1971). Metachromatic reactions of methylene blue when bound to various acidic polysaccharides show specific properties dependent upon the structure of the polymer (see M-NC-6, 1969, 1970). The latter technique demonstrated that kappa-Carrageenate assumes a net, right-handed helical conformation in solution while the closely related lambda-Carrageenate chain from the same organism assumes an opposite handedness in solution. The selectivity towards right- or left-handed twisting was shown to depend upon substituent groups on various polysaccharide chains which can even be identical in their glycosidic linkages. Those with a sulfate group in the C_4 of the 3-1 linked sugar show right-handed order while those with this group absent can assume left-handed chirality. Certain other features of primary structure are also correlated with this preference, i.e., a free hydroxyl group at the C_3 position of the 4-1 linked sugar (i.e., λ-carrageenate, heparin, kerato sulfate). a) Using both optical methods (uv C.D. and metachromatic reaction) it was shown that aminouronic acid moieties in bacterial exoglycosaminoglycans display uv Cotton effects similar to that of the amino sugar derivative and that the carboxyl groups (anionic sites) are not distributed in long runs along the polysaccharide chain. They occur, in part, in dimeric runs, however.

Scientific Significance: The molecular biology of GAG and complex polysaccharides is undergoing rapid growth. It is important to understand interrelationships among these biopolymers as well as to understand the biological activity of each separately. Recently computer analysis of possible conformations among these polymers based upon chain similarities have been reported by others who have jumped twice over a chasm in linking all their predictions to one X-ray crystallographic analysis. The findings reported here present experimental data which show that the predictions from the computer analysis were not correct and that molecular specificity depends largely upon substituent groups on neighboring sugar rings. The tendency for these chains to form single and multiple chain helical forms would be of great importance in their biological effects which involve a matrix environment for small cations and large proteins. The above flexibility in intra- and inter-molecular order would be important for rapid changing in membrane matrices. b) The acidic fibrous exopolysaccharide from Achromobacter georgiopolitanum is a useful model for the structure of GAG containing aminoglucuronic acid moieties and represents the molecular properties for gram-positive capsular polysaccharides (as opposed to gram-negative material which contains aminogalacuronic moieties). This material also resembles mammalian hyaluronic acid in its physical properties. The polysaccharide is shown to contain only moderate anionic density (greater than 10 Å apart) with a small and possibly variable percent of dimeric runs (i.e., carboxyls ~5 Å apart) so that specific regions of high negative density do not occur. Hyaluronic acid appears to have a similar metachromatic reaction indicating that a multichain helical order (proposed by others) is not important for these polymers in solution.

Proposed Course of Project: Specific optical measurements of GAG will be continued with continued structural and conformational studies on the various kinds of GAG gangliosides and other complex polysaccharides. Further studies on the interaction of these with biogenic amines will be pursued.

Honors and Awards:

Invited to membership in Biophysical Society in the new Biopolymer subgroup.
Referee for Biopolymers.
Referee for Journal of Molecular Biology
Referee for Analytical Biochemistry
Referee for Thrombosis Research

Publications:

Stone, A. L.: Helical conformations in acidic polysaccharides in solution. Biopolymers 11: 2625-2631, 1972.

Serial No. M-NC-7
1. Neurochemistry
2. Biophysical Chemistry
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of the Cell Membrane in Cellular Organization, A Molecular Study

Previous Serial Number: M-NC-7

Principal Investigator: David M. Neville, Jr.

Other Investigators: Hartmut Glossmann; Ronald Kahn and Ira Goldfine, NIAMD

Man Years

Total : 3.5
Professional: 2.5
Other : 1

Project Description:

Objectives: The aim of this project is to determine the specific chemical interactions which occur at the surfaces of cells which affect cellular differentiation and cellular organization. Specifically we propose that the recognition which cells have of their solid environment and neighboring cells is mediated by specific proteins on the surface membranes. In addition, the surface of each cell has protein or glycoprotein receptors for a variety of soluble hormones and transmitter substances. Our aim is to study the interaction between the receptor molecules and the soluble and fixed transmitter molecules so that the interaction which leads to altered cell function can be quantitatively defined. Having done this we propose to examine these interactions in pathological states in order to determine if altered receptors or receptor concentration play a role in disease states.

Methods Employed: Ultracentrifugation, disc electrophoresis, gel filtration, immuno-diffusion, fluorescence microscopy, circular dichroism, tissue and organ culture.

Major Findings: 1. The plasma membrane insulin receptor concentration on liver plasma membranes is markedly depressed under the following conditions: 1) hyperphagia induced by hypothalamic lesions with gold thioglucose; 2) obob mice fasted in the presence of high insulin dosage; 3) glucocorticoid treatment; 4) ACTH treatment; 5) growth hormone treatment. Each case is associated with insulin resistance. In the case of induced hyperphagia the receptor defect can be reversed by food restriction. There are two conclusions: 1) there is a good correlation of *in vivo* insulin resistance and *in vitro* loss of insulin receptor binding suggesting that the receptor concentration mediates these insulin resistant states; 2) the membrane receptor concentration is a variable entity under metabolic and hormonal control.

2. Phlorizin receptors are present in kidney brush border membranes and have a binding constant of dissociation in the same range as phlorizin concentrations which inhibit renal tubular reabsorption of glucose. Various sugars displace bound phlorizin to the degree to which they compete for the glucose carrier *in vivo*. The receptor is quite likely the glucose carrier.

Scientific Significance: 1. This is the first demonstration that the concentration of a surface membrane receptor can be varied by metabolic and hormonal events. It signifies a new mode of regulating the interaction of a cell with its environment. Cells are not required to react the same amount to a given stimulus but can vary their cell surface receptor concentration to increase or decrease their sensitivity to the environmental stimulus. This obviously has ramifications for neuropharmacology.

2. It now should be possible to isolate the glucose carrier using phlorizin binding as a marker.

Honors and Awards: None

Publications:

Kahn, C. R., Neville, D. M., Jr., Roth, J.: Insulin receptor interaction in the obese hyperglycemic mouse. *J. Biol. Chem.* 248: 244-250, 1973.

Kahn, C. R., Neville, D. M., Jr., Gorden, P., Freychet, P., and Roth, J.: Insulin receptor defect in insulin resistance studies in the obese hyperglycemic mouse. *Biochem. Biophys. Res. Commun.* 48: 135-142, 1972.

Goldfine, I. D., Gardner, J. P., and Neville, D. M., Jr.: Insulin action in isolated rat thymocytes. *J. Biol. Chem.* 247: 6919-6926, 1972.

Glossmann, H. and Neville, D. M., Jr.: Phlorizin receptors in isolated kidney brush border membranes. *J. Biol. Chem.* 247: 7779-7789, 1972.

Neville, D. M., Jr. and Kahn, C. R.: Isolation of plasma membranes for cell surface membrane receptor studies. In Laskin, A. I. and Last, J. A. (eds.): *Methods in Molecular Biology, Vol. 4, Subcellular Particles, Structures and Organelles*. New York, Marcel Dekker, Inc. In press.

Neville, D. M., Jr., Kahn, C. R., Soll, A. and Roth, J.: Plasma membrane insulin receptor regulation by genetic, metabolic, and hormonal factors. In *Protides of the Biological Fluids*, 21st Colloquium 1973, Pergamon Press, in press.

Serial No. M-NC-8
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biological and Biochemical Models for the Genetic Disease, Phenylketonuria (PKU)

Previous Serial Number: M-NC-8

Principal Investigator: Audrey L. Stone

Other Investigators: a) Seymour Kaufman; b) Elizabeth Neufeld, LBC, NIAMDD

Man Years

Total	:	1.2
Professional	:	0.6
Other	:	0.6

Project Description:

a) Development of a murine model for PKU using X-irradiated sires and a screening test for serum phenylalanine and tyrosine levels as a guide for heterozygosity for the phenylalanine hydroxylase gene; investigation of natural history of the enzyme with cell models, using murine, human and primate organs; search for mutants defective in the cofactor enzyme, dihydropteridine reductase (DPR). b) Development of human cell models for PKU studies using skin fibroblast and amnionic cell lines. Investigation of experimental lymphoid tissue and cell models.
Objectives: a) To produce and develop a mutant, murine strain with the genetic defect of PKU; to examine organs and cells of human, primate and other species for the expression of phenylalanine hydroxylase (PAH) activity; to compare in particular the biological and biochemical properties of phenylalanine hydroxylase from human liver and kidney cells and other tissues; to examine cofactor reductase as another possible mode of PAH deficiency.

b) Development of a prenatal diagnostic test for PKU homozygosity using amnionic cell lines and/or a postnatal diagnostic test for PKU using skin fibroblasts of lymphoid cell lines.

c) Elucidation of the biological range and function of dihydropteridine reductase in normal tissues and cell lines and in deficient mutants.

Methods: a) See M-NC-8, 1971 and M-NC-8, 1972(2); b) Fresh cell lines are grown to confluence in 250 ml flasks using various media. Cells are washed, then detached by trypsinization and harvested by low speed centrifugation, all at ambient temperature. Cells are washed and then lysed in 0.15 M KCl by three cycles of freeze-thaw. The soluble and sediment after centrifugation at 42,000 gravity hours are assayed for PAH, DPR and protein.

Major Findings: a) Four hundred suspect mice have been studied showing normal distributions for serum phenylalanine and tyrosine levels and for the ratio of phenylalanine to tyrosine. Both male and female distributions show plasma phenylalanine to be 0.082 mM ±0.023 mM; the ratio of phenylalanine to tyrosine is 1.03 ±0.27 for male and 1.08 ±0.33 for female distributions. The range and distribution of mouse plasma values and ratios is similar to that of the normal human distribution but ~0.02 mM and 0.2 lower in actual average values, respectively. Thus, the use of human heterozygote distribution values as a guide to predicting a possible mouse heterozygote appears to be valid. Of 5 offspring with high ratios (>1.5) only one gave a repeatedly high value of ~2.1. This suspect is female and infertile. Liver samples from 50 partial hepatectomies showed an average value of 0.223 ±0.045 units of DPR activity. Four offspring had values one-half to two-thirds normal and were considered suspect DPR mutants. These were all female, and infertile thus far. Furthermore, their plasma phenylalanine to tyrosine ratios were all high. b) Two cell lines (one adult; one neonate) were looked at for PAH and DPR expression. The adult line under specified conditions, showed approximately 20% of the PAH activity of human liver comparing tissue extracts per mg of protein. DPR activity was regularly found in all cell preparations in the order of magnitude of 10-100% of that found for the human liver. Attempts to increase (derepress) the level of PAH in the cell culture by varying the medium to very low tyrosine levels were unsuccessful in the two cell lines. However, DPR activity was higher when growth of an initial 48 hour transplantation in Eagles complete medium was continued to confluence in Waymouth synthetic medium. Certain modified assay procedures useful in stimulating increased PAH activity in rodent preparations were not stimulatory in these cell lines.

Scientific Significance: a) A screening program for a murine model for PKU has been shown to be possible. Time and numbers will provide the "mutant" offsprings for testing. No mutant strains have been developed to date, as expected from the very low statistical chance of success with 400 suspect mice. b) Initial promising results indicate that human fibroblasts contain measurable PAH activity. There is the possibility of a relationship between age of cell line and activity. Should normal amnionic cells or skin fibroblasts regularly show PAH activity with the analogous PKU cells negative, a technically simple test for PKU can be developed. c) New studies of DPR show that this enzyme may influence the relative levels of plasma phenylalanine and tyrosine. How this regulatory system intercepts that of PAH and whether it operates in a deleterious manner in PKU are new questions.

Proposed Course: a) The screening program for the murine model for PKU will be improved in statistical probability by the use of wild mice since this has become feasible at NIH (provided funds and personnel are available). b) The cell studies for a diagnostic test for PKU using amnionic or skin fibroblast will be completed. Studies with lymphoid cells will be continued as a model for studying the underlying mechanisms of regulation of PAH and DPR, as well as for possible clinical applications.

Honors and Awards:

Elected to membership in the American Society of Biological Chemists

Publications: None

Serial No. M-NC-9
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Conversion of Tryptophan to 5-Hydroxytryptophan

Previous Serial Number: M-NC-9

Principal Investigator: Seymour Kaufman

Other Investigators: A. Hanson Kappelman, Jeffry Tong

Man Years

Total :	1.7
Professional	0.7
Other	1.0

Project Description:

Objectives: The conversion of tryptophan to 5-hydroxytryptophan is believed to be the rate-limiting step in the biosynthesis of the neurotransmitter, serotonin. The reaction is catalyzed by the mixed-function oxidase, tryptophan hydroxylase. The goal of this project is to isolate the enzyme in order to study its regulatory properties.

Methods Employed: Standard methods of protein purification, including salt fractionation, ion-exchange chromatography, and sephadex-gel filtration, have been used.

Major Findings: We have continued to explore various fractionation procedures that might be useful for the further purification of this hydroxylase from brain. One important observation that was made is that the enzyme undergoes a protein concentration-dependent aggregation. This property of the enzyme will determine the types of fractionation steps that can be employed.

With the partially purified enzyme we have shown that the product of the reaction, 5-hydroxytryptophan, is a moderately potent inhibitor of the enzyme in the presence of tetrahydrobiopterin. The enzyme is much less sensitive to inhibition in the presence of the model cofactor, 6,7-dimethyltetrahydropterin ($DMPH_4$). In contrast to the product of the reaction, the end product of the biosynthetic pathway, serotonin, is a poor inhibitor of the hydroxylase.

Scientific Significance: The question of whether tryptophan hydroxylase might be regulated by product inhibition has been the subject of controversy. Previous *in vitro* tests of this possibility were all carried out in the presence of $DMPH_4$ and all showed that neither 5-hydroxytryptophan nor serotonin could inhibit, except at very high concentrations. By contrast, there is some evidence that this type of inhibition might be operative *in vivo*. Our observations with the purified enzyme may have supplied the answer to this puzzle.

The greater inhibition of the enzyme in the presence of tetrahydrobiopterin is another important illustration of the generality of the principle established from work in this laboratory: that for all of the pterin-dependent hydroxylases, substrates are better substrates and inhibitors (including the substrates themselves at high concentration) are better inhibitors in the presence of tetrahydrobiopterin than they are in the presence of model cofactors.

Proposed Course: Further purification of the enzyme will be attempted.

Honors and Awards: None

Publications:

Friedman, P. A., Kappelman, A. H. and Kaufman, S.: Partial purification and characterization of tryptophan hydroxylase from rabbit hindbrain. *J. Biol. Chem.* 247: 4165-4173, 1972.

Serial No. M-NP-3
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Reports
July 1, 1972 through June 30, 1973

Project Title: Electrical, ionic and physical properties of muscle

Previous Serial Number: Same

Principal Investigators: S. I. Rapoport and J. Vergara

Other Investigators: J. Bidinger, R. FitzHugh

Cooperating Units: Section on Technical Development
National Institute of Mental Health
(Mr. James Bryan)

Laboratory of Biophysics
National Institute of Neurological Diseases and Stroke

Man Years:

Total:	2.60
Professional:	1.00
Other:	1.60

Project Description:

Objectives: a) To determine the elastic and plastic properties of the muscle fiber membrane (sarcolemma) and relate them to the physiological function of the fiber, and to excitation-contraction coupling.

b) To study the effect of isometric stretch on muscle metabolism and muscle survival. To see whether effects on metabolism can be functionally related to effects on muscle survival, and to study fatigue in muscle.

Methods Employed: a) Photomicrographic techniques are used to study a single fiber under high magnification. The fiber is held by a transducer tension device, and measurements are made with usual amplification and recording techniques. Micromanipulation of the fiber is performed. Various mathematical techniques are used and analysis of data is made with the use of a computer. Theoretical models for elasticity of parts of the fiber are formulated and tested by analytic procedures.

b) Twitch and tetanic tensions are recorded from muscles with transducers, amplifiers and pen recorders. Muscles are stimulated periodically with standard neurophysiological techniques. Ionic determinations of Ca, Mg, Na and K are made after measuring wet and dry weights, ashing in a furnace, and using emission and atomic absorption spectrophotometry. Data are analyzed with computer techniques.

Major Findings: a) Tension and curvature of the sarcolemmal tube of the isolated frog muscle fiber were measured at different extensions and were used to calculate the anisotropic elastic properties of the sarcolemma, which is the surface membrane of the muscle fiber. A model was derived to obtain the four parameters of the elasticity matrix of the sarcolemma - a longitudinal and circumferential elastic (Young's) modulus and a longitudinal and circumferential Poisson ratio. On the basis of the calculated four parameters, it was possible to reconstruct the length-tension curve of the sarcolemma in the intact fiber and to estimate both the myofibrillar and the sarcolemmal contributions to intact fiber tension under different conditions of fiber extension. At sarcomere lengths less than 3µ, the sarcolemma makes no contribution to intact fiber tension. At higher sarcomere lengths, its contribution is of the order of 20%. Furthermore, the sarcolemma exerts a pressure on the myoplasmic contents due to a circumferential component of its tension, and this pressure was calculated for the first time in muscle from the data and model.

b) A 20-30% stretch of the sartorius muscle of the frog in a Ringer bath at room temperature retards the loss of contractility, the Na uptake and the loss of K that occur in unstretched muscles. Mg but not Ca content is higher in stretched than unstretched muscles. K content and contractility are roughly correlated under conditions of stretch and of soaking in different solutions - the higher the K, the higher the level of contractility. Ouabain, K free and cold Ringer solutions, conditions which inhibit the Na-K exchange pump, abolish the stretch effects on both contractility and ionic contents. Ouabain and K free Ringer accelerate, and cold retards the changes that occur in unstretched muscles in Ringer. These results suggest that stretch stimulates the Na pump, either directly, or indirectly through a stimulation of muscle metabolism, and that this stimulation is correlated with prolonged survival in vitro.

Significance to Bio-medical Research and Program of the Institute:

a) A knowledge of mechanical properties of the sarcolemma and myofibrils, and of their relative contribution to tension as a function of stretch, is required to understand the process of contraction and of the resistance of the muscle to stretch under physiological conditions. Correlation between electron microscopic structure of the sarcolemma and myofibrils and their properties permits a better understanding of the molecular forces regulating muscle contraction.

b) Stretch prolongs frog muscle survival and stimulates muscle metabolism. These effects have not been related up to now, but the present work shows that they may be related through the stimulation of the Na pump by stretch. The Na pump will determine ionic contents of the muscle, and these in turn may determine membrane potential and, therefore, contraction threshold. The effect of stretch in frog sartorius is relevant to the problem of stretch-induced muscle hypertrophy.

Proposed Course of Project: a) The work on sarcolemmal elasticity is complete and has been published in two recent papers.

b) The work on the stretch effect in muscle is being prepared for submission for publication. Research on this problem in the future will include consideration of muscle fatigue. It has been shown that muscles of dystrophic animals do not fatigue, and we shall try to determine at which step(s) in excitation-contraction coupling fatigue occurs.

Honors and Awards: None

Publications: Rapoport, S. I. The anisotropic elastic properties of the sarcolemma of the frog semitendinosus muscle fiber. Biophysical Journal, 13, 14-36, 1973. With Appendices by R. FitzHugh and S. I. Rapoport, and S. I. Rapoport and R. FitzHugh.

Serial No. M-NP-8
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Transport Mechanisms Across Membranes

Previous Serial Number: Same

Principal Investigators: S. I. Rapoport

Other Investigators: J. Bidinger, H. Thompson, D. S. Bachman, M. W. Brightman, T. S. Reese, M. Spatz, M. Hori, I. Klatzo and H. Levitan

Cooperating Units: (a) Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological Diseases and Stroke

(b) Laboratory of Brain Evolution and Behavior National Institute of Mental Health

(c) Department of Zoology, University of Maryland College Park, Maryland

Man Years:

Total:	1.35
Professional:	1.10
Other:	.25

Project Description:

Objectives: To consider the nature and function of the blood-brain barrier, and to consider and amplify the theory of active and passive transport across cellular membranes.

Methods: Surgical procedures are used for opening the skull and dura of rabbit and monkey, isolating and perfusing the internal or lingual arteries. Neurological examinations are performed, electroencephalograms used to determine brain function, and methods of perfusion and fixation for brain and eye histology are applied. Mathematics and the theory of irreversible thermodynamics are used to consider results quantitatively and to formulate and test a model for the osmotic action of substances on the blood-brain barriers. Computers are used for data analysis and model formulation. Electron microscopy is used to determine tracer passage.

Major Findings: (a) Theory and physiological and morphological demonstration of osmotic opening of the blood-brain barrier:The following work was done in collaboration with M. W. Brightman, T. S. Reese, M. Hori and I. Klatzo, and M. Spatz. The blood-brain barrier in the mammal can be osmotically opened in a reversible manner by shrinking cerebrovascular endothelial cells and separating the tight junctions that connect them. The tight junctions make the vascular endothelium act like a continuous cell layer which restricts exchange of large water soluble solutes and electrolytes between blood and brain. The application of concentrated solutions of salts, urea, lactamide and other slightly lipid soluble nonelectrolytes to the surface of the brain in rabbits opens up the barrier reversibly at the pia-arachnoid vessels. Injection of these substances into the internal carotid artery leads to reversible opening of the cerebrovascular endothelium over the whole cerebral cortex of one hemisphere. The theory of osmotic opening, which I had proposed on the basis of previous experiments, (Rapoport, S.I., Amer. J. Physiol., 219, 270, 1970 and Rapoport, S. I., Intern. J. Neurosci., 2, 1, 1971) was extended and confirmed by electron microscopy, using peroxidase as an electron dense tracer. An abstract of this work has been published: Rapoport, S. I., M. W. Brightman and T. S. Reese. Reversible osmotic opening of the blood-brain barrier (BBB) by opening tight junctions of cerebrovascular endothelium. Abst. 17th Ann. Meeting Biophysical Society, 13, 230a, 1973. Furthermore, the theory and applications were elaborated in an invited address I gave at the International Conference on Pharmacology and Pharmacokinetics, October, 1972, Fogarty International Center, which should be published eventually.

(b) Effect of osmotic opening of the blood-brain barrier on long term survival and neurological function in the monkey. This work is being done in collaboration with D. S. Bachman and H. K. Thompson. When we used the classical method in the monkey for perfusing hypertonic solutions into the brain (Steinwall, Acta Radiol., 49, 281, 1958), we found that when the blood-brain barrier opened there was associated neuronal damage with clinical signs of motor weakness in the animals just recovered. This might imply that opening the blood-brain barrier itself leads to brain damage in chronic experiments, and would restrict the eventual use of osmotic opening as a pharmacological tool. We guessed that the permanent ligation of the common carotid artery in the Steinwall procedure caused the neuronal damage, and not osmotic opening itself. We therefore developed a new perfusion method, perfusing solutions through the lingual artery when clamping temporarily the external and common carotids. The results changed dramatically, and about 90% of the monkeys with osmotic barrier opening had no obvious neurological damage. These latter observations have been published in abstract form; Rapoport, S. I. and Thompson, H. K. Osmotic opening of the blood-brain barrier (BBB) in the monkey without associated neurological deficits. Neurology, April, 1972.

(c) Equiosmolal opening of the blood-brain barrier by X-ray contrast media used in carotid arteriography. This work is being done in collaboration with J. Bidinger and H. K. Thompson. Each of Six X-ray contrast media of differing composition were perfused into the internal carotid artery of rabbits for 20 seconds, each opened the blood-brain barrier at a mean osmotic threshold of 1.2 osmolal, not different from the threshold osmolality for NaCl. The approximately equiosmolal thresholds for barrier opening by the X-ray contrast media and by NaCl suggests that the contrast media open the tight junctions between cerebrovascular endothelial cells, probably by osmotically shrinking the cells themselves.

(d) Dependence of neurotoxicity of X-ray contrast media on lipid solubility. This work is entirely theoretical at present and is being done in collaboration with H. Levitan. When I recognized that the X-ray contrast media opened up the barrier osmotically at equiosmolal concentrations, it became obvious that a factor other than osmotic barrier opening determined neurotoxicity. Neurotoxicity can differ markedly from one medium to another. We rank ordered clinical neurotoxicity of 8 X-ray contrast media and calculated their olive oil/water partition coefficients, which are classical parameters of lipid solubility. The correlation between lipid solubility and neurotoxicity was significant at the $P < 0.05$ level. We proposed that the more lipid soluble the agent, the faster it can enter the brain across the blood-brain barrier, which has permeability properties of the cerebrovascular endothelial cell membranes themselves. An abstract of this work will be published: Rapoport, S. I. and H. Levitan. Dependence of neurotoxicity of X-ray contrast media on lipid solubility. Ann. Meeting Amer. Soc. Neuroradiology, Boston, April, 1973.

Significance to Bio-medical Research and Program of the Institute:

a) The blood-brain barrier regulates exchange of materials between blood and brain and therefore controls brain metabolism and is important in brain pharmacology, since some drugs are excluded by it from entering the brain. The osmotic opening is the first demonstration of reversible barrier opening that is based on morphology and simple physical-chemical laws applicable to cells in general. It will permit experimental modification of the blood-brain barrier for studying brain metabolism and perhaps for pharmacotherapy.

b) X-ray contrast media are used by the method of intracarotid injection to visualize the cerebrovascular tree for clinical disorders of the brain. There is a significant morbidity and mortality associated with the technique, yet the factors affecting neurotoxicity are poorly understood. This work shows that (a) these agents can and probably do open the blood-brain barrier because of their high osmolality, and (b) that even if they do not open the barrier, they can enter the brain and affect neurones by virtue of their lipid solubility properties. This is the first time that neurotoxicity of X-ray contrast media is considered in terms of simple physical-chemical laws of osmosis and cell membrane permeability, and it would be hoped that less toxic contrast media (with lower osmolalities and lipid solubilities) will be synthesized in the future.

Proposed Course of Project: The theory and method of osmotic opening of the blood-brain barrier will be elaborated, and long term effects of barrier opening will be studied. The neurotoxicity of X-ray contrast media will be considered in more detail.

Honors and Awards:

(1) Invited address, "Target organ modification in pharmacology: reversible osmotic opening of the blood-brain barrier by opening of tight junctions," International Conference on Pharmacology and Pharmacokinetics, October, 1972. Fogarty International Center, Bethesda, Maryland.

(2) Invited address, "Relationship between metabolism and active transport in living cells," Symposium on Models for Transport in Biological Systems, Annual Meeting of American Association for Advancement of Science, December, 1972; Washington, D.C.

(3) Invited address, "Reversible opening of the blood-brain barrier," Second Head Injury Center Research Workshop, N.I.N.D.S., February, 1973, Bethesda, Maryland.

(4) Invited Lecture, University of Maryland, Department of Zoology, December, 1972: "A Theory of Active Transport."

(5) Invited Lecture, Case-Western Reserve School of Medicine, February, 1973: "The Nature of the Blood-Brain Barrier."

Publications:

Rapoport, S. I., M. Hori and I. Klatzo. Testing of a hypothesis for osmotic opening of the blood-brain barrier. Amer. J. Physiol. 223, 323-331, 1972.

Rapoport, S. I., D. S. Bachman and H. K. Thompson. Chronic effects of osmotic opening of the blood-brain barrier in the monkey. Science, 176, 1243-1245, 1972.

Spatz, M., Z. M. Rap, S. I. Rapoport and I. Klatzo. The effects of hypertonic urea on the blood-brain barrier and on glucose transport in the brain. In: Steroids and Brain Edema, H. J. Reulen and K. Schurman, eds. Springer-Verlag, Heidelberg, 1972.

Serial No. M-NP-47
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The ionic and metabolic basis of neuronal thermosensitivity

Previous Serial Number: Same

Principal Investigators: David O. Carpenter and Frederick-Karl Pierau

Other Investigators: None

Cooperating Units: None

Many Years:

Total: 1.75
Professional: 1.30
Other: .45

Project Description:

Objectives: This project is concerned with an investigation of a variety of membrane properties of neurons, particularly those properties which are temperature dependent and influence neuronal excitability. The ultimate aim is to understand the ionic mechanism underlying thermosensitivity and thermoregulation in the mammalian nervous system.

Methods Employed: Studies were carried out on he isolated nervous systems of the marine molluscs, *Aplysia* and *Loligo*, peripheral afferent fibers of rat, and in cultural cells. In the invertebrates intracellular recordings were made from neurons isolated from the animal where sea water composition and temperature could be manipulated and drugs added. In rats activity of afferent fibers was monitored from filaments of peripheral nerve upon changing skin temperature. Cultured cells were recorded with intracellular electrodes during temperature changes of the bathing solution.

Major Findings: 1) In *Aplysia* neurons thermosensitivity is imparted to each cell by at least two separate mechanisms. The first, an electrogenic sodium pump, causes the cell membrane potential to increase with temperature. The second mechanism tends to depolarize and excite the cell with warming as a result of a greater temperature dependence of the passive permeability of the membrane to sodium than to potassium. Similar results are obtained on neurons from squid ganglia.

2) The ionic basis of pacemaker discharge in Aplysia neurons. The endogenous pacemaker activity can be adequately explained by a model

containing a voltage and time varying potassium conductance in the face of a high resting sodium conductance. Metabolic processes influence discharge dramatically but are not directly responsible for discharge. The electrogenic sodium pump does not underlie the bursting discharge characteristic of some neurons, as has been suggested by others. A metabolic regulation of membrane conductance is important, but only in maintenance of a high membrane resistance which is necessary for normal pacemaker discharge.

3) Three types of acetylcholine response occur in Aplysia neurons. We have studied the ability of α bungarotoxin to block these three types of responses, due to Na^+, Cl^- and K^+ conductances respectively. All responses are blocked reversibly by the toxin and α bungarotoxin will bind to a membrane homogenate.

4) Collaborative studies with the Department of Physiology, Centro de Investigacion del Instituto Polytechnico Nacional, Mexico City, D. F., Mexico. This study on tht organization of primary afferent depolarization in the isolated spinal cord of the frog is a continuation of the program initiated in January, 1971, when Dr. Carpenter was a Visiting Professor in the Department of Physiology. The results of this study have appeared (J. Physiol. 1973).

Significance to Bio-medical Research and Program of the Institute: 1) An understanding of the elementary ionic and metabolic mechanisms of nerve cells is essential not only in the development of an understanding of how the brain works but also in pursuit of rational approaches to treatment of neuronal diseases by drugs. These experiments attempt to study these elementary processes in simple nervous systems and then to test the conclusions in mammalian nervous systems. In the case of neuronal thermosensitivity we now have isolated several temperature dependent processes which we will test in mammalian preparations.

2) The study of the ionic basis of neuronal pacemaker discharge also has relevance to the mammal. The neuronal pacemaker functions in a manner very similar to that of the heart. In addition, there is accumulating a considerable body of evidence suggesting that there may be endogenous activity of neurons in vertebrates as well as invertebrates.

Proposed Course of Project: These projects are concluded with the departure of this laboratory from NIMH.

Honors and Awards: None

Publications:

1. Carpenter, D. O. Ionic mechanisms and models of endogenous discharge of Aplysia neurons. Proc. Symp. on Neurobiology of Invertebrates, Tihany, Hungary, 1971, p. 11-33, published in 1973 by The Hungarian Academy of Sciences.

2. Carpenter, D. O. Electrogenic Na^+ pump and high specific resistance in nerve cell bodies of the squid. Science, 179: 1337-1338, 1973.

3. Carpenter, D. O. and P. Rudomin. The organization of primary afferent depolarization in the isolated spinal cord of the frog. J. Physiol. 229: 471-493, 1973.

Serial No. M-NP-48
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland 20014

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The state of ions and water in living cells

Previous Serial Number: Same

Principal Investigators: M. Hovey, D. O. Carpenter and A. Bak

Other Investigators: Harry K. Thompson

Cooperating Units: None

Many Years:

Total:	.85
Professional:	.85
Other:	0

Project Description:

Objectives: In this study we attempt to determine the state of water and ions in large nerve and muscle cells by measurements of the electrical conductivity of the internal medium.

Methods Employed: Intracellular conductivity was measured with a capacitance-conductance meter consisting principally of an operational amplifier and feedback resistance circuit with a high frequency source. Metal microelectrodes were supported by a micro positioner and electrically connected to the meter. Each electrode was calibrated in normal sea water. By diluting the sea water in steps (1 to 100% of normal) a calibration curve was drawn and compared with the records taken from the cells.

Major Findings: We have previously established that this technique measures conductivity of the solution at the tip of the microelectrode. We have extended the tests of the technique to an analysis of frequency of the applied current both in solutions and in neurons. In the neuronal cell bodies of the marine molluscs, Aplysia, Navanax and Anisodoris, the internal conductivity is only 5% that of sea water or less. In contrast, in the giant axon of the squid, internal conductivity is 80-100% that of sea water. In the giant axon of the marine annelid, Myxicola, internal conductivity is 30-50% that of sea water, and in the muscle fibers of the giant barnacle, internal conductivity is about 15% that of sea water.

Analysis of conductivity has also been made in solutions containing a known concentration of salt and a variety of organic substances. Addition of albumin, sugars and polysaccrides depress conductivity, but do not do so by binding the ions since the ionic activities are not depressed. Thus, it seems likely that these substances depress conductivity as a result of a structuring of cell water. By analogy we attribute the low internal conductivity of neuronal cell bodies to the existence of extensive structuring of cell water. In contrast little structured water appears to exist in squid axon, while the Myxicola axon and barnacle fibers show intermediate values of conductivity and thus presumably a lesser degree of structuring than is characteristic of cell bodies.

Significance to Bio-medical Research and Program of the Institute: Our results to date suggest that ions in nerve cell bodies exist in a very different state from those in axons. If this be true it is necessary to re-examine many of the concepts which were developed to explain electrical events in axons and which have been assumed to apply to all excitable tissues on the assumption that all ions are free. Moreover, it seems very possible that the state of ions in most cell bodies may be more like that in Aplysia neurons than that in squid axons since the cell bodies contain many more membraneous structures and a higher concentration of organic molecules (which are probably the site of the ion and water binding). Our observations, therefore, may have relevance to all types of cells.

Proposed Course of Project: This project is terminated with the departure of the laboratory from the National Institute of Mental Health.

Honors and Awards: None

Publications:

M. M. Hovey, A. F. Bak and D. O. Carpenter. Low internal conductivity of Aplysia neuronal somata. Science, 176: 1329-1331, 1972.

Serial No. M-NP-63
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The functional organization of the sensorimotor cortex in the initiation and control of movement

Previous Serial Number: Same

Principal Investigator: Edward V. Evarts

Other Investigators: Jun Tanji

Cooperating Units: None

Man Years:

Total:	4.55
Professional:	2.25
Other:	2.30

Project Description:

Objectives: This project is aimed at understanding how the inputs and outputs of the cerebral sensorimotor cortex function in control of movement and to discover what areas of the cortex initiate movement, what areas control and regulate movement, and how these areas for initiation and control are interrelated.

Methods Employed: Microelectrodes are used to record the activity of individual neurons during natural, voluntary movement in the monkey. This use of single unit analysis in relation to voluntary movement is analogous to the long-established use of single unit analysis in determining how the brain receives and elaborates its sensory input, but single unit analysis has only recently been carried out in animals trained to perform skilled movements during recording.

Major Findings: Effects of Behavioral Set and Expectancy on Activity of Neurons in Sensorimotor Cortex of the Monkey. In recent years there has been increasing debate as to the fundamental organizational pattern of the sensorimotor cortex. According to one view, activity generated by stimulation of peripheral receptors would pass in relatively unadulterated form to the sensory division of the sensorimotor cortex from whence it would flow to other cerebral structures where decisions would be made as to the behavioral responses to the input, following which information would be relayed to the motor cortex where the signals associated with voluntary movement would be generated. An alternative model has been favored by Wall, who believes that expectancy and "set" can modify input signals even prior to their arrival at the level of the sensory division of the sensorimotor cortex. Wall has written that primary sensory systems may function to control the action of convergent pathways rather than to feed into conscious decision-making centers. The availability of techniques for recording activity of neurons in intact animals has now made it possible to assess the way in which the expectancy and set modifies input-output relations in the nervous system.

In the experiment on the neuronal correlates of set, monkeys were trained to carefully maintain a certain hand position and then, shortly before the hand was perturbed, were given an "instruction" as to how they should respond to the forthcoming hand perturbation when it occurred. The "instruction" was communicated visually, as the appearance of a red light or a green light. The red light signalled to the monkey that he should pull toward himself when the perturbation occurred, and the green light meant that he should push away when the perturbation occurred. Two different sorts of perturbation were used, and there were thus four different stimulus-response sequences. There have been two major findings. First of all, it has been found that neurons in both postcentral and precentral zones of sensorimotor cortex show changes of activity as a result of the "instruction" and prior to any direct input from hand receptors or output to hand musculature. Thus, when a light comes on instructing the animal as to whether he should push or pull later when his hand is perturbed, nerve cells of both postcentral and precentral cortex assume a discharge frequency (or "set") which is differential depending on which of the two instructions has been given. This change in sensorimotor cortex activity occurs even though there is no overt behavioral response to the instructions. Thus, there is a striking correlate between the set, attitude, or expectancy of the animal and discharge of neurons in a part of the brain which will ultimately serve to carry out the instruction. According to older views, it would have been believed that activity of neurons in sensory and motor areas would change only in association with a change in peripheral sensory input or a change in motor output. In these studies, however, changes were seen both in postcentral sensory and precentral motor divisions of the sensorimotor cortex when the animal "preset" his sensorimotor apparatus in a particular way.

A second finding concerns changes in the neuronal activity generated in sensorimotor cortex by the perturbation of the hand. As already pointed out, monkeys were trained to respond to the perturbation of the hand in one of two ways, the nature of the response depending on the prior "instruction". But in addition to two sorts of instructions, there were two sorts of perturbation of the hand, and it was found that the cortical neuronal response to the hand perturbation differed markedly depending upon the prior instruction. The difference in neuronal response as a function of instruction and "set" was present at extremely short latencies from the instant of the perturbation, latencies so short as to lead the normal activity set up by the perturbation to be referred to as a "reflex". The finding that the set, attitude, or expectancy of the subject can so profoundly modify the "reflex" response is of especial interest in view of the fact that the process was one resulting from learning.

Significance to Bio-medical Research and the Program of the Institute: The finding that nerve cells in sensorimotor cortex undergo specific changes as a function of central set and expectancy provides insight into the neuronal factors which may mediate selective attention, purposive behavior, and learning. The neuronal events which have been seen must involve changes in parts of the nervous system anatomically close to primary receptors, and this fact allows us to focus attention on a limited anatomical zone in seeking to discover where the changes may be taking place in association with modifications of behavior based on learning.

Proposed Course of Project: The finding that the learned set of an animal can so profoundly modify patterns of activity in sensorimotor cortex leads us to try to localize the particular sites in the nervous system where these modifications may take place. Does modification of neuronal response to a peripheral stimulus take place as a result of changes in the thalamus or in the spinal cord? If we can find the site and then locate the particular neuronal processes which give rise to it, we will have made great advances in understanding the cerebral mechanisms underlying learning and selective attention.

Honors and Awards:

1. Intensive Study Program, Neurosciences Research Program, Boulder, Colorado, July 24-August 11, 1972.

2. Visiting Neuroscience Speakers Program, Dept. of Anatomy, University of Wisconsin, Madison, Wisconsin, May 7, 1973.

Serves on:

1. Board of Co-editors, Experimental Brain Research
2. Advisory Board, Journal of Neuroscience
3. Editorial Board, International Review of Neurobiology
4. Council, Society for Neuroscience
5. Secretary, Society for Neuroscience

Publications:

Evarts, E.V.: Activity of thalamic and cortical neurons in relation to learned movement in the monkey. Internatl. J. Neurol. 8: 321-326, 1971

Evarts, E.V.: Activity of motor cortex neurons in association with learned movement. Internatl. J. Neurosci. 3: 113-124, 1972

Evarts, E.V.: Studies on cerebral mechanisms of motor control. Internatl. J. Neurosci. 4: 89-92, 1972

Evarts, E.V.: Motor cortex reflexes associated with learned movement. Science 179: 501-503, 1973

Serial No. M-NP-65
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Functional role of the basal ganglia in the control of movement and posture

Previous Serial Number: None

Principal Investigator: Mahlon R. DeLong

Other Investigators: Peter L. Strick and William T. Burriss

Cooperating Units: None

Man Years:		
	Total:	3.60
	Professional:	2.55
	Other:	1.05

Project Description:

Objectives: This study is directed towards learning how the basal ganglia function in the control of movement and posture. What is the functional organization of these structures? Do they play a role primarily in the initiation of movement or in feedback control? What are the specific contributions of these nuclei as contrasted with those of other brain structures involved in motor function, such as the cerebellum?

Methods Employed: These studies utilize the techniques of single unit recording in intact moving animals developed in this Laboratory over past years. Animals are trained to make movements of the limbs, and cell activity is recorded during the animal's performance of the task. Cell activity is correlated with various aspects of the movements.

Major Findings: Clinico-pathologic studies in man have long indicated that the corpus striatum (i.e., caudate-putamen and pallidum) plays an important role in the control of movement and posture, since when these structures are damaged through disease, abnormalities in posture, muscle tone, and involuntary movements frequently result (e.g., Parkinsonism, chorea, etc.). These large subcortical nuclei, forming a major portion of the so-called extrapyramidal motor system, are of special interest from the standpoint of motor control since the major influences from these nuclei appear to be exerted upon the motor cortex. The major efferents from the corpus striatum arise from the pallidum and terminate in "motor" portions of the thalamus (i.e., the ventrolateral and ventroanterior nuclei), which in turn project to the motor cortex. Previous experimental studies dealing with the functions of the corpus striatum have been concerned largely with the effects of ablation and stimulation of portions of these structures. These approaches have not proven particularly revealing, due in part to the "distance" of these structures from the "output" and the difficulties of confining lesions and stimulation to these deeply located and anatomically complex regions. It was thus of considerable importance to apply the techniques of single unit recording in intact animals in order to learn more about how these nuclei normally function during movement.

Recently, Kornhuber has interpreted clinical and experimental evidence to indicate that the major motor function of the basal ganglia is to generate slow ("ramp") movements, whereas the function of the cerebellum is to preprogram and initiate rapid ("ballistic") movements. If this view is correct, one might predict that the activity of basal ganglia neurons would be preferentially related to slow rather than rapid movements. This hypothesis has been tested experimentally by recording the activity of neurons in the putamen in a monkey during the performance of a motor task involving both slow and rapid arm movements. It was found that more than half of the units in the putamen discharged solely or preferentially in relation to slow movements while less than 10% of units discharged preferentially during rapid arm movements. This finding indicates that at least a portion of the basal ganglia (the putamen) is primarily involved in the control of slow movements and provides some experimental support to the notion of Kornhuber that the primary motor function of the basal ganglia is to generate slow rather than rapid movements. However, since previous studies (as described in last years annual report) have indicated that neurons in the globus pallidus are involved in the initiation of rapid arm movements, and since numerous putamen units in the present study discharged in relation to rapid as well as slow movements, it is clear that the basal ganglia do not function solely in the control of slow movements. One of the major points of the Kornhuber hypothesis is that the basal ganglia actually initiate the slow movements, i.e., the activity of neurons in these structures occurs prior to the onset of the movement and before other motor regions such as the motor cortex, thalamus or cerebellum. This point was not resolved by this study since precise measurements of determinations of the earliest change in muscle activity associated with the ramp movements were not made in this study.

Significance to Bio-medical Research and the Program of the Institute: These findings on the preferential relation of neural activity in the putamen to slow movements may have relevance to the pathophysiology of movement disorders involving the basal ganglia in man. Considerable evidence points to a selective disturbance in slow movements in many of these disorders. Generally speaking, the involuntary movements associated with basal ganglia dysfunction (e.g., chorea, athetosis, dystonia) are "slow" in character. Also, in the akinetic syndrome of Parkinsonism the major difficulty appears to be in the initiation of slow movements, such as rising from a chair or bed, turning, walking, etc. The initiation of simple rapid movements in Parkinsonism patients appears to be less impaired than one would expect.

Kornhuber has suggested that akinesia might result from loss of the ramp-generating mechanisms of the basal ganglia, whereas rigidity, chorea, athetosis and dystonia might result from various forms of "release" of these same neural mechanisms. The present experiment, indicating that the activity of a large portion of the striatum is preferentially involved in the control of slow movements, is consistent with this view as well as the evidence for involvement of the striatum in the pathogenesis of akinesia as well as rigidity, chorea, athetosis and dystonia.

Studies on the function of the corpus-striatum are of relevance to the program of the Institute since these regions of the brain appear to have important integrative as well as purely motor functions which, when disturbed as in disease, produce disturbances of movement, motivation, and affect. Current theories suggest that the site of action of L-Dopa, which abolishes symptoms in the majority of Parkinsonian patients, is upon the cells of the caudate and putamen via a dopaminergic nigro-striatal pathway. It has been suggested as well that disturbances in dopamine metabolism within corpus striatum and/or the histologically indistinguishable nucleus accumbens are involved in the pathogensis of schizophrenia, since enhancement of dopamine turnover in the brain seems to be the only distinguishing feature of the neuroleptics antipsycholic drugs (such as chlorpromazine and haloperidol). This enhancement of turnover of Dopamine within the striatum appears to result from blockade of dopamine receptors. An understanding of the normal functioning of the corpus striatum should provide a firm basis for studies on the effects of disease and pharmacologic agents on these structures.

Proposed Course of Project: Experiments now in progress are concerned with the question of (1) timing of the activity in basal ganglia in relation to the onset of movements and (2) the role of other motor centers (motor cortex, thalamus, and cerebellum) in the control of slow and rapid movements. Recordings just completed from arm area of the motor cortex indicate that the majority of cells there are related to both slow and rapid arm movements in a manner similar to the muscles of the arm. It moreover appears that unit activity in basal ganglia indeed does preced the earliest changes in muscle activity associated with the task, which is consistent with the view of Kornhuber that the basal ganglia are involved in the initiation of slow movements. Recordings will be carried out in the cerebellum and thalamus in the near future.

Honors and Awards: None

Publications:

M. R. DeLong. Putamen: Activity of single units during slow and rapid arm movements. Science, 179: 1240-1242, 1973.

M. R. DeLong. Motor Functions of the Basal Ganglia: Single unit activity during movement. In: The Neurosciences: 3rd study program (In Press).

Serial No. M-NP-66
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Reports
July 1, 1972 through June 30, 1973

Project Title: The role of the cerebellum and vestibular nuclei in the generation of saccadic eye movements

Previous Serial Number: None

Principal Investigator: F. A. Miles

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.40
Professional:	1.80
Other:	.60

Project Description:

Objectives: Experiments have been concerned with the role of the cerebellum and vestibular nuclei in the programming of rapid (saccadic) eye movements.

Methods Employed: The approach used here involved microelectrode recordings, initially in the cerebellum, more recently in the vestibular nuclei, in conscious monkeys, and concentrated on individual units whose firing patterns correlated with the animal's saccadic eye movements. Each animal was trained to press a bar which switched on a small spot of light on a screen facing him and to release the bar only after the light dimmed. If the animal succeeded in releasing the bar within 1/2 sec. of this dimming, then he was rewarded with a drop of water; all of the animal's fluid intake was earned by working at this task. Using this approach, monkeys were trained to fixate a small spot of light and maintain this fixation even if the position of the spot was changed. Thus, it was possible to induce the animal to generate saccadic eye movements of known magnitude and direction merely by changing the position of the fixation target. This behavioural task requires immense vigilance from the animal and cannot be performed with peripheral vision, hence successful performance could only be achieved by the animal if he made the required eye movements. Oculograms recorded from implanted electrodes confirmed the nature of the animal's eye movements whilst he was performing the task and were used as a response trigger for data display: correlations between eye movements and individual neurone firing patterns were made on-line by using a raster display in which each action potential was represented as a spot on the screen of a storage oscilloscope; the oculogram signals generated by the eye movements were used to initiate sweeps of the oscilloscope and the pulses representing action potentials were displayed after passing through a delay line. In this way, unit firing patterns during successive saccade trials were displayed in rows on the storage oscilloscope; the delay facility allowed one to observe the unit firing before, as well as during and after, the eye movements. A major advantage of this form of display is that it readily reveals not only the nature of the correlation between eye position and unit firing but also its timing and consistency.

Upon isolating an eye-movement correlated unit, the animal was induced to make a variety of eye movements with various amplitudes, directions, and start positions. In the initial part of the study, recordings were made from lobules VI and VII in the cerebellar vermal cortex and the fastigial and interposed cerebellar nuclei. Very few neurons recorded from these areas showed a strong correlation with eye movements--only 20 out of several hundred investigated. Most of these units generated a burst in association with the eye movement, which could precede the movement by up to 10 msec. Usually it correlated with ipsilateral saccades, but occasional units generated such bursts during all saccades. The duration of the burst related to the amplitude of the saccade but only loosely, usually being longer for larger saccades. The initial start position seemed to be irrelevant in determining the characteristics of this firing.

The difficulty in finding saccade-related units in the cerebellum mitigated against any extensive analysis of the cerebellum's role in eye movements with this approach. It was therefore decided to probe the vestibular nuclei, whose possible involvement in programming saccadic eye movements had long been postulated.

Major Findings: The single unit recordings in the vestibular nuclei revealed that the activity of a large proportion of the cells in the medial and superior vestibular nuclei is very closely correlated with eye movements. Some neurones fire in relation to the position of the eyes in the orbits, others only in relation to the movement of the eyes, and yet others show both relationships; occasional units show maintained firing which is suppressed during all eye movements. Thus, each saccade is accompanied by a step, pulse, or pulse-step change in the firing of individual neurones. The precision of this coupling between single neurone behaviour and eye movements is especially impressive and the vestibular nuclei are clearly in possession of very accurate information about the oculomotor programmes. In some units the earliest changes in firing precede the eye movement by 10-20 msec, whilst in others they occur during the movement. Many neurons are strongly directional, firing only in relation to movements of the eyes to one or other side of the orbits, whilst others--especially the pulse-type units--often fire in relation to all eye movements.

Significance to Bio-medical Research and the Program of the Institute:From work in other laboratories, a great deal is now known about the firing patterns of oculomotor neurons in conscious monkeys, each saccade being the result of a very precisely programmed pulse-step increase in the activity of neurones supplying agonist muscles and a converse pulse-step decrease in those innervating antagonists. It is most interesting that the present study should reveal that the individual components of the saccadic programme (the transient pulse and the step change in firing) are represented in the vestibular nuclei, a finding consistent with the earlier mentioned idea that these eye-movement programmes are "compiled" in the vestibular nuclei. Since we now know that the oculomotor firing is continuously undergoing modification as a result of vestibular inputs generated by head movements, it is perhaps not unreasonable to expect the vestibular system to be intimately involved in the programming. Experiments are now under way which it is felt will help to clarify the neural mechanisms involved in computing the saccadic programme and in modifying it to compensate for movements of the head.

Proposed Course of Project: The next part of the project will seek to look more directly at vestibular-ocular stabilisation by attempting to characterize the nature of any vestibular influence on the neurones in the medial and superior vestibular nuclei, especially those which have been seen to correlate with eye movements when the head was fixed, i.e., when there was no vestibular input. It is proposed to approach this problem in two ways. Firstly, a vestibular input will be provided by rotating the whole animal about a vertical axis with a special servomechanism; during this vestibular stimulation, the animal will fixate either on a distant stationary target (involving vestibular-ocular compensation) or on a target which oscillates with him (involving a vestibular input but no eye movements); the latter situation requires that the monkey suppress the vestibular-ocular reflex in order to maintain stable fixation (--an exercise the bus passenger must also perform if he wishes to read his newspaper); such techniques should make it possible to identify vestibular contributions to neuronal activity and perhaps help to establish exactly where in the system the suppression of the vestibular influence normally occurs. Secondly, the animal's head will be free to move and he will be trained to elicit a coordinated pattern of eye-head movements; in this situation, the head can be stopped at any time in any position by operating an electrical clutch; thus, it will be possible to interrupt the normal head movement and, as a result, establish more precisely the nature of the vestibular component; the millisecond precision with which it is possible to time the neural events tied to this sudden vestibular input hopefully may provide a latency classification which will help to indicate the individual neurone's position in the vestibular-ocular processing network. It is hoped that single unit recording techniques employed in these diverse behavioural situations will help to reveal operational aspects of vestibular-ocular stabilisation.

Honors and Awards: Invited to give a paper at the VIth International Meeting of Neurobiologists in Oxford, England, September, 1972.

Publications:

1. F. A. Miles. Centrifugal Control of the Avian Retina. I. Receptive field properties of retinal ganglion cells. Brain Research, 48: 65-92, 1972.

2. F. A. Miles. Centrifugal Control of the Avian Retina. II. Receptive field properties of cells in the isthmo-optic nucleus. *Brain Research*, *48*: 93-113, 1972.

3. F. A. Miles. Centrifugal Control of the Avian Retina. III. Effects of electrical stimulation of the isthmo-optic tract on the receptive field properties of retinal ganglion cells. *Brain Research*, *48*: 115-129, 1972.

4. F. A. Miles. Centrifugal Control of the Avian Retina. IV. Effects of reversible cold block of the isthmo-optic tract on the receptive field properties of cells in the retina and isthmo-optic nucleus. *Brain Research*, *48*: 131-145, 1972.

5. L. J. Rogers and F. A. Miles. Centrifugal Control of the Avian Retina. V. Effects of lesions of the isthmo-optic nucleus on visual behaviour. *Brain Research*, *48*: 147-156, 1972.

Serial No. M-NP-68
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the neuronal activity of the prefrontal cortex

Previous Serial Number: Same

Principal Investigator: Hiroaki Niki

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.65
Professional:	1.90
Other:	.75

Project Description:

Objectives: While there exists a considerable body of evidence suggesting the involvement of the dorsolateral prefrontal cortex in spatial delayed response performance, little information is available about the neuronal correlates of this task performance. In the preceding year the unit activity of the prefrontal cortex was analyzed during the delayed alternation performance (DA) and several types of related units were found in the middle part of the principal sulcus. It is noteworthy that half of these related units responded differentially in relation to the direction of movement (right or left) and 5% of total units recorded showed a different discharge rate between right and left trials during the delay period of DA. The aim of the subsequent experiments is to analyze further the nature of directional specificity found in the first experiment.

Methods Employed: Single unit activity of the dorsolateral prefrontal cortex is analyzed during the delayed alternation using a method developed by Dr. E. V. Evarts.

Major Findings: Directional specificity at the time of responding was found to be related to an absolute stimulus position, while the specificity during delay was related to a relative position of the stimulus. Directional specificity during delay was seen only in DA and not in cue-guided button pressing control task.

Significance to Bio-medical Research: The prefrontal cortex has been thought to be concerned in higher mental processes, but the way cells of this area participate in such processes has not been well understood. Thus, an understanding of the physiology of the prefrontal cortex is essential for an understanding of the cerebral basis of mental processes.

Proposed Course of Project: In a subsequent study, unit activity of the other part of the prefrontal cortex (periarcuate area and orbitofrontal area) will be investigated in other tasks such as conditional discrimination and go-no-go type discrimination in order to differentiate a functional role within the prefrontal cortex.

Honors and Awards: None

Publications: None

Serial No. M-NP-69
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Reports
July 1, 1972 through June 30, 1973

Project Title: Influence of centrally-acting drugs on motor cortex activity during attentive behavior

Previous Serial Number: None

Principal Investigators: Allan F. Mirsky and Jesus B. Otero

Other Investigators: None

Cooperating Units: Division of Psychiatry
Boston University
School of Medicine
Boston, Massachusetts

Man Years:

Total:	2.85
Professional:	2.25
Other:	.60

Project Description:

Objectives: The overall goal of this research is to understand the central nervous system alterations occurring during impaired attentive behavior. Previous work has investigated behavioral and EEG aspects of impaired attention produced in trained monkey subjects by the administration of secobarbital and chlorpromazine (cf Bakay Pragay, E. and Mirsky, A. F.: The nature of performance deficit under secobarbital and chlorpromazine in the monkey. Psychopharmacologia (Berl.) 28, 73-85, 1973). This work suggested that the mechanism of impairment produced by these two drugs differed; chlorpromazine seemed to produce primarily a phasic blocking of input process subserving arousal. Secobarbital, on the other hand, seems to suppress the functional level not only of arousal processes but of motor effector mechanisms as well. The present study was designed to focus on these effector mechanisms as they come into play during the performance of a sustained discriminated attention task. The question being investigated is whether and how neuronal activity is altered by centrally-acting drugs during attentive behavior. The methods used permit dissection of two components of the motor behavior: time to initiate the response after the "go" signal has appeared (Initiation Time) and time to execute the response (Movement Time).

Methods Employed: Monkey subjects are trained to perform a go-no go color discrimination task for liquid reinforcement. Recordings of extracellular motor neuron activity and EEG are obtained during the performance of the task, and selected doses of centrally-acting drugs are administered. The intent is to monitor the activity of task-related neurons both prior to and after drug administration. Ideally, single neurons will be followed during a complete cycle of drug action until behavioral recovery (i.e., pre-drug performance levels) is observed.

Major Findings: Data collection is still in a preliminary stage; therefore findings must be viewed as tentative. However, from observation of 12-15 neurons monitored during drug (secobarbital) administration the following observations can be noted:

1. As in the case of the prior study, significant increments in Initiation Time (IT) are observed, paralleling the degree of omission error occurrence.

2. Paradoxically, in most experiments, Movement Time (MT) is significantly decreased while IT is increased. Both IT and MT tend to return to normal levels upon recovery.

3. The pattern of neuronal firing parallels closely the change in IT; i.e., if IT is slowed by 250 msec., the pattern of firing (although preserved) is delayed by about the same amount. The normal latencies tend to return on recovery from drug action.

4. The base rate of the majority of units decreases during barbiturate administration; however, some units fire more rapidly.

Significance to Bio-medical Research and the Program of the Institute: The information obtained from this project is relevant to two questions of interest to the Institute: (1) the symptom of attention impairment is prominent in several diseases of largely unknown pathophysiology, i.e., schizophrenia and petit mal epilepsy. Further, it is a commonly observed characteristic of hyperkinetic children. Any information concerning alterations in central nervous system activity during impaired attention should aid in the understanding of the possible brain pathology which is associated with these clinical problems. (2) Although heroin abuse receives more attention, barbiturate abuse is probably more widespread and more dangerous from a physiological point of view. Information concerning the neural effects of barbiturates and related compounds is needed to aid in the comprehension of this problem.

Proposed Course of Project: Additional animals will be prepared for study of barbiturate effects on neuronal activity. The work will be extended, as well, to chlorpromazine, a drug of great current clinical interest as a "major tranquilizer." It is intended to characterize and contrast the action of these two drugs at the motor unit neural level during attentive performance. The expectation is that chlorpromazine will produce less effect at the level of the motor cortex than does secobarbital and that the action that is seen will tend to be more phasic and less sustained.

Honors and Awards: None

Publications: None

Serial No. M-NP-70
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neurochemical and neuroanatomical correlates of behavior

Previous Serial Number: None

Principal Investigator: Edward V. Evarts

Other Investigators: Rufus Gunn

Cooperating Units: None

Man Years:

Total:	0.60
Professional:	0.60
Other:	-

Project Description:

Objectives: This project is aimed at obtaining anatomical and chemical correlates of changes in neuronal activity occurring in association with behavior. Other projects in the laboratory have focused on discovering the way in which individual nerve cells change their discharge patterns in association with attention, learning, memory, and movement, and have found that certain regions of the brain are involved differentially in certain specific sorts of behavior. Microelectrode recording techniques are valuable from the standpoint of establishing such changes in activity, but are limited in the sense that the number of nerve cells which can be examined is necessarily small in relation to the total number involved. Furthermore, microelectrode recording techniques selectively obtain activity from large cells. It is thus important to study the cerebral correlates of behavior in a way which provides additional information. The present project seeks to obtain this additional information by applying neuroanatomical and neurochemical techniques. The first method to be employed in this project will be one which has been worked out in the Laboratory of Cerebral Metabolism by Louis Sokoloff and his associates. This method allows metabolic activity in localized cerebral regions to be determined. The development of this method has been completed by Sokoloff, and we must now examine the way in which different cerebral regions show modifications of metabolic activity in association with different forms of behavior. If selective changes in metabolic activity are shown by Sokoloff's method, we will use electron microscopy to isolate the cellular components which participate in the observed changes of activity.

Major Findings: During the first year, the major work on the project has been of a technical nature, setting up the equipment to be used in obtaining electron microscopic sections of the brain. An electron microscope acquired last year has been put into operation and good sections are now being obtained. In the technique which we will employ it is important for us to maintain precise knowledge of where within a section of brain the minute particle of tissue examined in the electron microscope has come from. Excellent progress has been made on setting up procedures which will guarantee accurate correlative information along these lines.

Significance to Bio-Medical Research and the Program of the Institute: The new project is one which is aimed at bridging the gap between several currently successful programs already underway in the NIMH research program. The project seeks to provide a link between the Laboratories of Cerebral Metabolism, Neurophysiology, and ultimately Neuropharmacology. Work in the Laboratory of Cerebral Metabolism has resulted in the discovery of methods which will pinpoint selective changes in metabolic activity of the brain. Work in the Laboratory of Neurophysiology has resulted in discoveries as to selective changes in neuronal activity in association with behavior. It is hoped that by combining the methods of these laboratories, we will be able to obtain more information on the relationship between cerebral metabolism and behavior.

Proposed Course of Project: Much technical development remains to be done in connection with this project, and this will involve considerable training, both for investigators and technical personnel involved in the project. It therefore seems likely that for the second year of the project as well as the first, most of the activity will involve setting up methods and making sure they are functioning properly.

Honors and Awards: None

Publications: None

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